



Everaert, N., Van Cruchten, S., Weström, B., Bailey, M., Van Ginneken, C., Thymann, T., & Pieper, R. (2017). A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis. *Animal Feed Science and Technology*. <https://doi.org/10.1016/j.anifeedsci.2017.06.011>

Peer reviewed version

Link to published version (if available):  
[10.1016/j.anifeedsci.2017.06.011](https://doi.org/10.1016/j.anifeedsci.2017.06.011)

[Link to publication record in Explore Bristol Research](#)  
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Elsevier at <http://www.sciencedirect.com/science/article/pii/S037784011631063X>. Please refer to any applicable terms of use of the publisher.

## University of Bristol - Explore Bristol Research

### General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:  
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

# **A review on early gut maturation and colonization in pigs, including biological and dietary factors affecting gut homeostasis.**

Nadia Everaert<sup>1</sup>, Steven Van Cruchten<sup>2</sup>, Björn Weström<sup>3</sup>, Michael Bailey<sup>4</sup>, Chris Van Ginneken<sup>2</sup>, Thomas Thymann<sup>5</sup>, Robert Pieper<sup>6</sup>

<sup>1</sup>Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, TERRA Teaching and Research Centre, University of Liège, Passage de Déportés 2, Gembloux, Belgium.  
[Nadia.everaert@ulg.ac.be](mailto:Nadia.everaert@ulg.ac.be)

<sup>2</sup>Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium, [steven.vancruchten@uantwerpen.be](mailto:steven.vancruchten@uantwerpen.be); [chris.vanginneken@uantwerpen.be](mailto:chris.vanginneken@uantwerpen.be)

<sup>3</sup> Department of Biology, Lund University, Sölvegatan 35, SE-223 62 Lund, Sweden, [bjorn.westrom@biol.lu.se](mailto:bjorn.westrom@biol.lu.se)

<sup>4</sup>School of Veterinary Science, University of Bristol, UK, [Mick.Bailey@bristol.ac.uk](mailto:Mick.Bailey@bristol.ac.uk)

<sup>5</sup> Comparative Pediatrics and Nutrition, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, [thomas.thymann@sund.ku.dk](mailto:thomas.thymann@sund.ku.dk)

<sup>6</sup> Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Königin-Luise-Strasse 49, 14195 Berlin, Germany, [robert.pieper@fu-berlin.de](mailto:robert.pieper@fu-berlin.de)

Abbreviated title: Neonatal gut development

Key words: weaning, microbiota, immune system, digestive physiology, intrauterine growth restriction, prematurity

Corresponding author: Nadia Everaert [nadia.everaert@ulg.ac.be](mailto:nadia.everaert@ulg.ac.be)

## **Abstract (400 words max)**

During the prenatal, neonatal and post-weaning periods, the mammalian gastrointestinal tract undergoes various morphological and physiological changes alongside with an expansion of the immune system and microbial ecosystem. This review focuses on the time period before weaning and summarizes the current knowledge regarding i) structural and functional aspects ii) the development of the immune system, and iii) the establishment of the gut ecosystem of the porcine intestine. Structural and functional maturation of the gastrointestinal tract gradually progress with age. In the neonatal period colostrum induces gut closure, leads to an increase in intestinal weight, absorptive area and brush border enzyme activities. During the first weeks of life, an increased secretion of stomach and pancreatic enzymes and an increased uptake of monosaccharides and amino acids are observed. The development in digestive function coincides with development in both the adaptive and innate immune system. This secures a balanced immune response to the ingested milk-derived macromolecules, and colonizing bacteria. Husbandry and dietary interventions in early life appear to affect the development of multiple components of the mucosal immune system. Furthermore, the composition of the intestinal microbial communities seems to be affected by the early postnatal environment, which might also contribute to gut maturation, metabolic and immune development. Understanding the interplay between morphological, functional and immunological maturation, as influenced by early microbial colonization and ingestion of dietary factors, is of utmost importance to identify management and feeding strategies to optimize intestinal health. We discuss some possible implications related to intrauterine growth restriction, and preterm delivery as these both dramatically increase the risk of mortality and morbidity. In addition, some nutritional interventions during the perinatal period in both sows and piglets will be discussed in the light of possible health consequences early in life and later on.

47

48 Abbreviations:

49 GD: days of gestation

50 GIT: gastrointestinal tract

51 HP: high protein

52 IUGR: intrauterine growth restriction

53 MMC: migrating myoelectric complex

54 NEC: necrotizing enterocolitis

55 PND: postnatal days

56 PP: Peyer's patches

57 SCFA: short chain fatty acids

58 scFOS: short-chain fructooligosaccharides

59 SWE: seaweed extract

## Introduction

The development of the mammalian intestine is driven by intrinsic (e.g. ontogenetically programmed) and extrinsic (e.g. nutrients, microbiota) factors during the prenatal, the neonatal and the post-weaning period (Buddington and Sangild, 2011). In pigs, it is well established that weaning causes substantial changes in the intestinal structure, microbial composition and intestinal functional properties (e.g. Pluske et al., 1997; Montagne et al., 2007). In current pig production, an abrupt weaning process usually is imposed between 3 to 5 weeks of age, leading to increased susceptibility to intestinal dysfunction from which putative pathogens such as *Escherichia coli*, *Clostridium perfringens* can take advantage and cause intestinal disease (Ewing and Tucker, 2008). Understanding the factors that disturb development of the gastrointestinal tract (GIT) in the pre-weaning period, may be important to better understand physiological and immunological reactions and the susceptibility to gut disorders around weaning and later in life. This review specifically summarizes the current knowledge regarding i) structural and functional aspects ii) the development of the immune system, iii) the establishment of the gut ecosystem in early life. Regarding the development of the intrinsic neuro-endocrine system and its role in modulating the structural and functional maturation of the gastrointestinal tract, we refer to other reviews (Van Ginneken, 2012; Thymann, 2016). An overview of important developmental changes after birth is provided in Table 1. In addition, some factors disturbing or modulating gut colonization and maturation (i.e. preterm birth, intrauterine growth restriction, nutritional interventions) will be discussed in the light of possible health consequences early in life and later on.

### 1. Structural and functional aspects of early life gut maturation

The GIT, i.e. the stomach, small intestine and large intestine, originates from the primitive gut during embryonic development and is composed of 4 distinct layers, i.e. the tunica mucosa, tela submucosa, tunica muscularis and tunica serosa. Although all layers are present in the stomach,

small and large intestine, distinct spatiotemporal differences in morphology occur in these 3 parts of the GIT during development (Van Ginneken et al., 2002), which is also reflected in their functional capacity (Henning et al., 1994). In pigs enterocytes with a high endocytotic activity (fetal-type) are gradually replaced during the first 3 weeks of life by new adult-type cells with markedly reduced endocytotic activity (Smith and Peacock, 1980, Klein, 1989). Additionally, this shift in enterocyte types occurs concomitantly with the marked shift in expression of the enterocyte brush border disaccharidases with decreased lactase and increased sucrase and maltase activities with age (Le Huërou-Luron, 2002). These maturational changes gradually progress with age, but become most evident at weaning, when the gut digestive and absorptive capacity rapidly adapts to effectively utilize the weaning diet instead of the easily digestible milk diet. In addition, the stomach acid secretion amplifies and besides an increased production of gut enzymes an elevated pancreatic function is realised (Cranwell, 1995).

## 1.1. Development of the gastrointestinal tract's structure

### 1.1.1. Stomach

The gastric corpus and mucosal layer of the cardiac and fundic region show the most pronounced alterations during development (Xu et al. 1992). The fundus region of the stomach of the pig shows 3 periods of intensive growth, i.e. around the 3<sup>rd</sup> month of gestation, around birth and between postnatal days (PND) 10 and 20. At about 45 days of gestation (GD), gastric pits develop and around the 3<sup>rd</sup> month of gestation fundic glands are formed. The parietal cells will be the first ones to differentiate at approximately GD60, whereas the mucous neck cells and chief cells take up to the 3<sup>rd</sup> month of gestation for differentiation although mucous secretion can already be observed around GD45-50 (Georgieva and Gerov, 1975a). As such, these differences have consequences for the onset of the digestive enzymes and hormones secretion (see section 1.2.1). At birth, hypertrophy and hyperplasia realize the massive growth of the

stomach, whereas later - during the first weeks after birth - hyperplasia is the key driver for growth (Lindemann et al., 1986).

#### 1.1.2. Small intestine

From GD40 onwards, villi are observed in the largest part of the small intestine of the developing piglet, the jejunum. The crypts and muscularis mucosae layer are formed around the 3<sup>rd</sup> month of gestation. By then epithelial cells lining the lumen also differentiate into enterocytes, goblet cells and enteroendocrine cells (Georgieva and Gerov, 1975b; Van Ginneken et al., 2001, Willemen et al., 2012; Willemen et al., 2013). The presence of Paneth cells in pigs is still under debate (Burkey et al., 2009). However, they might only be found around birth (Georgieva and Gerov, 1975b). Towards the end of gestation, the small intestine will grow more rapidly than the body itself, resulting in an increase of its relative weight by 70-80% during the last 3 weeks of gestation (McPherson et al., 2004). After birth, enteral nutrition and bioactive substances in the colostrum and milk stimulate intestinal mucosal proliferation and facilitate the gut closure (Takeda et al., 2004) (see also 2.). This results in an increase of the relative volume of the tunica mucosa after birth, whereas the relative volume of the tela submucosa and tunica muscularis decreases (Van Ginneken et al., 2002, Van Ginneken and Weyns, 2004). The intestinal epithelium is unique because cell proliferation, cell differentiation and apoptosis occur in an ordered manner along the crypt-villus axis. Cell proliferation occurs mainly in the crypts, whereas the villus houses the differentiating and differentiated cells. Subsequently cells die by apoptosis towards the villus tip in adult animals (Hall et al., 1994, Yen and Wright, 2006). These mechanisms allow the small intestine to maintain a relatively constant cell number (Hall et al., 1994, Potten, 1997). However, another pattern is observed in neonatal piglets. In these young animals, apoptotic cells are present along the entire length of the villi and cells seem to die in groups (Biernat et al., 2003, Godlewski et al., 2005). Furthermore, an enhanced mitosis and a significant decline in apoptosis rate is present during the first few days after birth (Wolinski et al., 2003, Godlewski et al., 2007), which is reflected in the postnatal enlargement of the intestinal

mucosa and increase in villus length. Nevertheless, this intestinal epithelial cell turnover is a dynamic process that is markedly affected by nutritional status and specific nutrients in the diet (see further) (Carver and Barness, 1996; Ziegler et al., 1999; Ziegler et al., 2003).

During development regional differences in morphology - which reflect slightly different functions - appear along the small intestine. In a 'mature' small intestine, the length of the villi increases from the duodenum to the mid jejunum but decreases again towards the ileum (Skrzypek et al., 2005; Van Ginneken et al., 2002; Wiyaporn et al., 2013); villi in the duodenum and jejunum have a more regular shape in comparison to in the ileum (Skrzypek et al., 2005), and crypts are usually deeper in the proximal part compared to in the middle and distal parts of the small intestine (Oste et al., 2010). Furthermore, villus height and shape change with ageing. At birth the small intestinal mucosa is lined with finger-like villi (Skrzypek et al., 2010). During the first days after birth, the length of the intestinal villi quickly increases from approximately 200 to 300  $\mu\text{m}$  at birth up to  $>500 \mu\text{m}$  at 3 days of age (Skrzypek et al., 2010). After 3 days of age the length of the villi decreases and at the same time the villus diameter increases, resulting in leaf-like shaped villi (Cera et al., 1988). In neonates, intestinal crypt depth is lower than in 3-day old piglets, whereas crypt depth again decreases when the piglet becomes older (Skrzypek et al., 2005). In these morphological changes colostrum (see 4.3.1) plays an essential role. Feeding colostrum leads to an increase in intestinal weight, absorptive area and brush border enzyme activities (see section 1.2.2) (Wang et al., 1996; Zhang et al., 1997). Moreover the presence of milk-based nutrients continue to affect gut maturation since Huygelen et al. showed it resulted in crypt deepening and cell proliferation (Huygelen et al., 2014).

### 1.1.3. Large intestine

The colonic epithelium is still immature at birth (Montedonico et al., 2006). At PND1, the weight of the large intestine increases by approximately 30% and by PND3 its weight has doubled by a contribution of all layers (Bach and Carey, 1994). In this respect, the proximal colon of the neonate looks similar to the small intestine, i.e. villus-like structures, with a not yet defined



function, are transiently observed in the proximal large intestine the first days after birth (Cremaschi et al., 1979; Xu et al., 1992a). They were presumably also present during late gestation. This morphological similarity with the small intestine is also accompanied with functional similarities as these colon enterocytes can transport amino acids until 2 weeks after birth (Xu et al., 1992). Most probably these neonatal colon enterocytes are gradually replaced by newly synthesized colon enterocytes that lack this ability (Sepulveda and Smith 1979). The decline in this transport function is independent of age at birth but seems to be affected by the neurohormonal status of the animal and food passage (Wooding et al., 1978).

## 1.2. Development of the gastrointestinal tract's basic functions

### 1.2.1. Stomach enzymes and secretion

Gastric acid secretion is low at birth but increases rapidly during the first week of life as the parietal cells increase in size and number (Xu and Cranwell, 1990, Sangild et al., 1992) and maximal acid secretion is reached from 5-6 weeks of age (Cranwell, 1995). The gastric proteases show a characteristic shift in their expression during development linked to the nutrition of the young pig (Sangild et al., 1991): chymosin, having primary milk clotting function and low proteolytic activity. After birth, the chymosin concentration declines steadily up to 3-4 weeks of age and after 2 months of age chymosin activity is undetectable. Instead, pepsinogen A, only found in traces at birth, gradually increases, take over and becomes the main protease (together with gastricsin) in the fundic mucosa from 4-5 weeks of age (Sangild et al. 1991). In addition to piglet age, creep feeding before weaning and weaning to solid feed increase the gastric acid and the protease secretion capacity of the stomach (Cranwell, 1985).

### 1.2.2. Intestinal brush border enzymes

When looking at the activities of the brush border peptidases during the suckling period, the overall trend is that the activities are relatively high at birth and then decrease with age in

suckling pigs (Le Huerou-Luron, 2002). The brush border carbohydrases in the small intestinal enterocytes do not follow the same pattern as the peptidases and moreover develop differently after birth (Le Huerou-Luron 2002). Lactase activity, which cleaves milk lactose, undergoes a marked decrease during the first 2-5 weeks of life. In contrast, from one week of age, the activity of both maltase and sucrase markedly increase. These changes of brush border disaccharidase activity seem to be substrate-independent and ontogenetically programmed. For example, pigs fed a formula with lactose as sole carbohydrate source (50% of diet dry matter) already showed reduced lactase activity and increasing maltase activity at 2 weeks of life (Pieper et al., 2016a).

### 1.2.3. Intestinal absorption and intestinal closure

Uptake of macromolecules to the enterocytes by endocytosis is a feature of the foetal and/or pre-weaning periods of mammals (Brambell, 1970; Baintner, 1986). In the enterocytes, internalised macromolecules are broken down in large digestive vacuoles, formed after coalescence with lysosomes, and are used in the metabolism or further transferred in undegraded form into the general circulation. Although the transfer of passive immunity (immunoglobulin G) is most important, other macromolecules, like hormones and growth factors, are transferred from mother to offspring during the pre-closure period (Sanderson and Walker, 1993). Additionally this mechanism could aid in, the surveillance of dietary and microbial macromolecules (antigens) in the gut after their absorption and exposure to the immune system may be also important, especially for tolerance induction. This non-selective absorption is further facilitated by a low degradative capability in the intestinal lumen, due to a low secretion of pancreatic enzymes (Pierzynowski et al., 1995), and the presence of proteinase inhibitors from colostrum and piglet blood plasma (Weström et al., 1985), thus enhancing the absorption to as much as 50-80 % of the amount fed. The high transfer of colostrum-derived macromolecules into blood, however, ceases abruptly 18-36 h after birth during intestinal closure. This results in an exclusion of molecules with a molecular weight greater than a few

kilo-daltons, while smaller molecules are absorbed independently of the closure process (Weström et al., 1984). In case of deprivation of colostrum intake or starvation, intestinal closure can be delayed and uptake of proteins is prolonged (Payne et al., 1962; Lecce et al., 1973).

The small intestinal transport mechanisms for the end products of the digestion, i.e., monosaccharides, amino acids or peptides (di- and tri-peptides) and fat digestion products (e.g., fatty acids and monoglycerides) and their development have been described earlier (Buddington and Malo, 1996, Buddington et al., 2001).

After closure, the uptake of macromolecules into the enterocytes will continue for some time, but there is no further transmission to the blood and the macromolecules will remain in the enterocytes to be degraded or to finally disappear from the mucosa due to cell shedding at the villus tip. These fetal-type enterocytes will be gradually replaced by new adult-type cells having low endocytotic activity (Smith and Peacock, 1980). This cell replacement proceeds in a proximal-distal direction along the intestine, being completed in the distal part by the time the pigs are 3-4 weeks of age. Although the mechanism involved in the induction of closure remains obscure, components present in colostrum and humoral factors released in response to feeding have been implicated (Ekström et al., 1988). Nevertheless, these adult type cells have a more efficient enzymatic brush border membrane machinery resulting in an increase in rate of uptake of monosaccharides and amino acids during the suckling period (see also 1.2.2.). There is limited information available about whether these adult type enterocytes can absorb macromolecules in the pig. The studies performed show that the transfer is low, but not insignificant, and could be enhanced by decreasing intestinal degradation (Svendsen et al., 1990). Results similar to pigs have been obtained in other species and it has become obvious that the absorption might be increased during injuries and inflammatory conditions in the intestines, opening up a paracellular pathway between the enterocytes for leakage of macromolecules (Sanderson and Walker, 1993).

242

#### 243 1.2.4. Gastrointestinal transit

244 Complex interactions between myogenic, neural and hormonal mechanisms determine the rate  
245 of gastric emptying and are associated with meal volume and content (Low, 1990; Olsson and  
246 Holmgren, 2011). In piglets, there is a rapid emptying of liquid nutrients (complete after 15 min)  
247 immediately after suckling. As the milk clots, a period of inhibition is established. This is then  
248 followed by a slow phase of emptying, representing the phase of clot hydrolysis, and liquefaction  
249 occurs (Decuypere et al., 1986). When the piglets reach an age of 4 to 6 weeks, 50 - 70% of the  
250 ingested milk empties within 1 h (Wangsness and Soroka, 1978; Moughan et al., 1991) and a  
251 total volume of 80 to 90% is expelled within 3 h (Moughan et al., 1991), although others  
252 reported that gastric emptying is already completed within 2 h (Kidder and Manners, 1968;  
253 Braude et al., 1970). Neonatal piglets, 2 to 6 days after parturition, show a similar pattern of  
254 gastric emptying (Wright et al., 1998). However, when using non-disintegrating radio-opaque  
255 pellets faster gastric emptying was observed in suckling piglets (PND21) compared to recently  
256 weaned piglets (Snoeck et al., 2004).

257 Nutrients and hormones also control intestinal motility, and thus transit, but the autonomic  
258 nervous system that includes extrinsic and intrinsic (enteric) pathways plays the most  
259 important role (Hansen, 2003; Olsson and Holmgren, 2011)(for review see Van Ginneken, 2012).  
260 In suckling piglets, no alterations in intestinal passage of barium sulphate were detected  
261 between PND7 and PND21 (Kidder and Manners, 1968). Similar observations were made in a  
262 study with Evans blue in PND0, PND3 and PND10 piglets (Huygelen et al., 2015). However, at  
263 PND28 the geometric center, a marker for intestinal transit (Miller et al., 1981), was higher than  
264 in the younger age groups, implying a faster small intestinal transit in weanling piglets  
265 (Huygelen et al., 2015).

266

## 267 2. Early-life development of the gut immune system

In the ungulate species, including the pig, no macromolecular passage between mother and offspring can take place during the fetal period, due to the epitheliochorial placenta consisting of four epithelial layers between the fetal and maternal blood circulations (Baintner, 1986). Consequently, the newborn piglet and neonates of the ungulate species are born hypogammaglobulinaemic and must acquire passive immunity (IgG) via mammary secretions for their survival (see also 1.2.3). Secondly, because the placenta of the pig is essentially impermeable to macromolecules in the absence of infection, most piglets are born essentially antigen-naïve and, as a consequence, the immune system is extremely poorly developed. Thus a quick maturation of the immune system is essential (Bailey et al., 2005).

## 2.1. Organised lymphoid tissues

In adult animals, Peyer's patches are clearly visible in the wall of the small intestine. In the pig, multiple discrete Peyer's patches occur throughout the jejunum, while a single, large patch extends from the ileocaecocolic junction for perhaps 1 meter through the ileum into the jejunum (Rothkotter and Pabst, 1989; Barman et al., 1997). These Peyer's patches are present at birth but are very difficult to identify without microscopic examination: at this stage they contain very small, primordial follicles and almost no T-cells (Makala et al., 2000). Within the first two weeks of life, expansion of the follicles occurs and the T-cell zones begin to be populated (Barman et al., 1997; Makala et al., 2000). However, while the size of B-cell and T-cell compartments expands, function remains limited for several weeks. In the neonate, immunoglobulin heavy chain gene rearrangements are much more restricted than in adults, and development to use the full range of adult V-segment genes does not occur until around six weeks (Sun et al., 1998; Wilson et al., 2007). Similarly, B-cells use primarily the mu, or IgM heavy chain for the first 6 weeks or so, and IgA positive follicles do not appear until about 6 weeks (Wilson et al., 2005). Thus, while piglets under 6 weeks can make antigen-specific responses, the quality of the response may be limited compared to older animals.

Studies in sheep have suggested that the ileal Peyer's patch may have a specific role in expanding the antibody repertoire in young animals, as the Bursa of Fabricius does in birds (Yasuda et al., 2006). In pigs, there are several features of the ileal patch which have suggested that the same may be true: one specific segment within the patch contains follicles but no T-cell zones and does not recruit lymphocytes from blood, similar to the bursa in chickens (Pabst et al., 1991). However, detailed analysis has found no evidence for repertoire diversification in early B-cells within this patch in the pig (Sinkora et al., 2011), and it has also been proposed that the ileal Peyer's patch may simply be the primary source of undiversified IgA antibodies (Butler et al., 2016).

## 2.2. Diffuse lymphoid tissues – the intestinal mucosa

In adult pigs, the intestinal mucosa is heavily infiltrated with multiple types of lymphocytes, apparently engaged in surveillance and maintenance of homeostasis rather than in expression of active immune responses. Although the mucosa is not considered an organized lymphoid structure, lymphocyte subsets clearly occupy distinct spatially environments and, presumably, co-operate within them. Within the epithelial layer, CD8alpha-positive T-cells predominate: most of these are CD8alpha/beta positive, true cytotoxic T-cells, but many are CD8alpha/alpha positive and include both unconventional T-cells and subsets which appear not to express T-cell receptors at all. Deep to the basement membrane of the villi are conventional CD4 T-helper cells, and these are mixed with antigen-presenting cells bearing the T-cell restriction molecule, MHC class II (Vega-Lopez et al., 1993). In the pig, the antigen-presenting cell population includes both conventional dendritic cells and capillary endothelium, and both appear to interact with CD4 T-cells (Wilson et al., 1996; Inman et al., 2010a). Beneath the villi, around the crypts, plasma cells secreting IgA and IgM are present, together with significant numbers of eosinophils.

Even less of this diffuse architecture is present in the newborn piglet than in the organized Peyer's patches. However, the cell types appear after birth in a well-ordered sequence rather

than all at once. Initially, antigen-presenting cells appear in the intestinal mucosa during the first two weeks of life. CD4 T-cells appear in the mucosa during weeks three and four, and CD8 T-cells in the epithelium start to appear from four to 6 weeks old, such that the normal architecture apparent in adult animals is not properly developed until 6 weeks after birth (Bianchi et al., 1992; Vega-Lopez et al., 1995). Even where the architecture appears normal, the interactions between cell types may be unusual in young animals: mucosal CD4 T-cells appear to interact both with resident dendritic cells and with capillary endothelial cells, whereas interactions in adults appear to be exclusively with dendritic cells (Inman et al., 2010a).

### 2.3. The influence of microbiota

Most of this expansion appears to be driven by microbial colonization. True antigen-naïve pigs are very hard to generate, since food contains intact molecules and may contain microbial products even when autoclaved or irradiated. Such animals have been reared but their immune systems have not been well characterised. However, several groups have reared germ-free piglets and compared them either with conventional animals or with defined-colonized animals. While some development of the mucosal immune system may occur in germ-free piglets, it is very limited compared to conventionals. Colonization with a defined, limited microbiome can recapitulate most of the development of antigen-presenting cell, B-cell and T-cell compartments which is apparent in conventional piglets (Sun et al., 1998; Inman et al., 2012). Thus, there is the potential for altered nutrition to change the rate at which the immune system develops, or to affect the types of cell which appear sequentially, or their interactions. Early, pre-weaning interventions in husbandry and diet have been shown to affect the establishment of intestinal microbiome and the development of the immune system, although the causal link between the two is difficult to establish directly. Piglets removed from the sow and reared in high containment units on bovine-based milk formula develop different microbiomes from their littermates on the sow and marked differences in the mucosal immune system in all three

compartments: antigen-presenting cells (more rapid recruitment), T-cells (fewer regulatory T-cells); and B-cells (increased antibody responses to weaning diet) (Inman et al., 2010b; Lewis et al., 2012). Similarly, piglets reared on indoor and outdoor farms develop different microbiomes and differences in expression of genes associated with MHC-dependent antigen presentation in the intestinal mucosa (Mulder et al., 2009; Schmidt et al., 2011; Mulder et al., 2011). Thus, husbandry and dietary interventions in early life do appear to affect the rate of development of multiple components of the mucosal immune system and provide potential targets for optimizing enteric health.

### 3. Early-life gut colonization and establishment of the gut ecosystem

The growing (weaned) and adult pig intestinal tract is colonized by highly diverse microbial consortia. Similar as in other mammalian species, factors such as host phylogenetic background, the early environment, and diet have likely been the major driving forces for the co-evolution of close microbe – host relationship (Ley et al., 2006). The adult pig gastrointestinal tract harbours likely more than 1,000 different bacterial species, of which most belong to the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinbacteria* and *Spirochaetes* (Kim et al., 2011; Ramayo-Caldas et al., 2016). Recent advances in metagenomic deep sequencing or comparative analysis of whole genome sequences have also increased our understanding about the metabolic potential of the porcine gut microbiome (Lamendella et al., 2011; Looft et al., 2014; Xiao et al., 2016). In addition, a high similarity between functional bacterial pathways between the human and the porcine gut microbiome was recently observed, supporting the potential use of pigs as model for humans (Xiao et al., 2016). The developing pig microbiome has recently also been linked with growth traits, thus opening translational potential for the pig industry (Ramayo-Caldas et al., 2016).

Although the establishment of the intestinal microbiome of pigs has yet not been studied in such detail and over longer periods as in humans, it is likely that similar patterns occur in dependence of certain life events such as environmental or dietary changes, or medical intervention. The



372 establishment of microbial communities in the human neonatal GIT undergoes several  
373 successional events from birth until adulthood – usually characterized by an overall increasing  
374 microbial diversity and activity (Koenig et al., 2011; Yatsunenکو et al., 2012). In the developing  
375 intestine, microbial succession continues until a point called ‘climax’ community is reached,  
376 where bacterial populations remain relatively stable over time and even return to their initial  
377 composition after perturbations (Detlefsen et al., 2007). Whether this concept can be  
378 transferred to pigs with a relatively short life span is unclear and data rather point towards an  
379 ongoing change in bacterial composition in growing pigs between the ages of 10 to 22 weeks  
380 (Kim et al., 2011). In pigs, only few studies exist regarding the colonization patterns very early  
381 (<14 days of age) in life. Apparently, the very early colonizers between birth and 2 days of age  
382 are mainly members of the genera *Escherichia*, *Clostridium*, *Fusobacterium*, *Streptococcus* and  
383 *Enterococcus*, whereas *Lactobacillus*, *Bacteroides*, *Prevotella* and *Ruminococcus* increase in  
384 abundance afterwards during undisturbed colonization processes (Bian et al., 2016; Kubasova et  
385 al., 2017). Similarly, *Clostridium difficile*, a putative pathogen associated with an increased risk  
386 for pre-weaning mortality in pigs, can be found in high numbers in the GIT of neonatal piglets  
387 but disappears at the age of approximately 14 days (Grzeskowiak et al., 2016). Although the  
388 early colonizing communities in pigs cannot be clearly linked to the intestinal microbiota of the  
389 sow (Bian et al., 2016; Kubasova et al., 2017), there are indications that the early postnatal  
390 environment has strong influence on the composition of the intestinal microbial communities  
391 later in life (Thompson et al., 2008; Schmidt et al., 2011; Starke et al., 2013). This early life  
392 “microbial programming” might be essential for gut maturation, metabolic and immune  
393 development later in life (Merrifield et al., 2015)(see also 2.3). Changing this early life  
394 environment (e.g. by moving piglets from their mother into artificial rearing units) leads to a  
395 different development of the intestinal microbiota (Schmidt et al., 2011). Feeding formula-based  
396 diets (e.g. in breeding lines with large litters of >14 piglets) may amplify these conditions and  
397 increase the risk for enteric disease. For example, a high level of lactose in the formula (by  
398 replacing maltodextrin) reduced the incidence of necrotizing enterocolitis, increased the

abundance of certain lactic acid bacteria in the small intestine, reduced the concentration of short chain fatty acids (SCFA) in the stomach and increased the concentration of lactate and SCFA in the large intestine (Thymann et al., 2009). With respect to the proliferation of potential harmful bacteria, some studies reported increased *C. perfringens*-like and *Streptococcus*-like phylotypes in the distal small intestine of formula-fed preterm pigs, and higher abundance of clostridia and coliforms in the colon (Siggers et al., 2008; Thymann et al., 2009). However, systematic studies about microbial succession in term-born and formula fed piglets are still scarce. A recent study showed that high levels of lactose (>40%) in formula for neonatal piglets exceeded the small intestinal digestive capacity and increased large intestinal concentration of fermentation metabolites almost 3-fold, and the abundance of enterobacteria and members of clostridial cluster I as compared to suckling piglets (Pieper et al., 2016b). A further understanding of the dynamics of early life intestinal microbial colonization in relation to dietary interventions may help to identify risk factors for intestinal disease and the possible need for medical interventions later in life.

#### 4. Lessons learned from suboptimal gut maturation and nutritional interventions

As the digestive tract of piglets undergoes structural, functional, immunological maturation and as the colonization interplays during these processes, several factors might impact these developmental changes. Dietary interventions can modulate all the piglets of one litter, but yet, not every piglet reacts on a certain event to the same extent. This can be partially explained by inter-individual differences in gut maturity. Hence, intra-uterine growth restriction and prematurity will be discussed first as factors affecting gut colonization and maturation, followed by an overview of dietary interventions.

##### 4.1. Intrauterine growth restriction

###### 4.1.1. Introduction

In comparison with other livestock animals, pigs exhibit the highest number of naturally occurring low birth weight pigs (Cooper, 1975). This low birth weight is mainly the result of intrauterine growth restriction (IUGR) most often caused by placental insufficiency (Ashworth et al., 2001). The prevalence of IUGR piglets increases in highly prolific sows due to uterine crowding and in gilts due to competition with the maternal resources for growth (De Vos et al., 2014). This high prevalence of IUGR pigs affects the profitability of pork production since these pigs exhibit higher mortality and morbidity rates, poorer growth rates and poorer carcass quality when compared with their normal littermates (Tuchscherer et al., 2000, Gondret et al., 2002, Quiniou and Gaudré, 2002, Bee, 2007, Beaulieu et al., 2010, Paredes et al., 2012). This poor profitability and high loss of piglets has driven research to explore the link between intrauterine impaired growth and gut maturation and colonization. Moreover, given the concept of 'metabolic programming', the long-term effects of intrauterine growth restriction gain importance.

#### 4.1.2. IUGR gut maturation and colonization

Many authors have reported differences in intestinal architecture between IUGR and normal birth weight pigs during the neonatal period. In these first days of life, the intestinal absorptive surface was smaller in IUGR as indicated by reduced intestinal villus height and crypt depth (Xu et al., 1994, Che et al., 2010, D'Inca et al., 2010, D'Inca et al. 2011, Mickiewicz et al., 2012, Ferenc et al., 2014). According to D'Inca et al. (2010) this reduction in surface area results from a disturbed proliferation-apoptosis homeostasis possibly in association with an altered gene expression pattern of growth-related proteins (Wang et al., 2005). The smaller intestinal surface area in IUGR piglets is reflected in diminished activities of brush border enzymes, especially lactase (Xu et al. 1994, Che et al. 2010, D'Inca et al. 2010, D'Inca et al. 2011, Ferenc et al. 2014), and affects the gut barrier function. In IUGR piglets, transcellular and paracellular permeability is transiently increased (Wang et al., 2015). The higher transcellular permeability is probably related to a delayed 'gut closure' (Sangild et al., 1999, Jensen et al., 2001) since IUGR piglets

ingest less colostrum (Amdi et al., 2013). Nonetheless, the increased paracellular permeability indicates a compromised barrier, and can explain the higher translocation of antigens and microorganisms in neonatal IUGR piglets as shown by D'Inca et al. (2011).

These structural and functional differences appear not to be present in IUGR piglets that have survived the first critical days after birth (Boudry et al., 2011, Huygelen et al., 2014, Mickiewicz et al., 2012, Wang et al., 2015), although proteomic analysis suggests a continuous impairment (Wang et al., 2010). Proteome analysis revealed that proteins involved in key biological processes, such as absorption, digestion, transport, apoptosis, metabolism and redox homeostasis are affected in IUGR piglets throughout the suckling period (D'Inca et al., 2010, Wang et al., 2010). The interplay between gut maturation and colonization is evident (see 3). Therefore it would not be unexpected to see differences in gut colonization in IUGR. In IUGR pigs the adherent bacterial flora contained a higher number of colony-forming units but only in the neonatal period (D'Inca et al., 2010). Thus, similar as the structural and functional characteristics of the intestinal epithelium, differences between IUGR and normal birth weight pigs with regard to gut colonization and composition are not present in IUGR piglets older than 1 week (D'Inca et al., 2010, De Vos et al., 2014, Prims et al., 2016). Nevertheless, newborn IUGR pigs that face difficulties in receiving sufficient nutrition are at higher risk of dying in the immediate postnatal period due to a compromised digestion, a failing barrier function, and an altered colonization. These factors can be held responsible for the higher morbidity and mortality rates in the neonatal period (Quiniou and Gaudré, 2002). IUGR piglets that receive sufficient support can catch up with the normal maturation pattern.

## 4.2. Prematurity

### 4.2.1. Introduction

Following conception the pig fetus develops and matures to reach full term. The number of days to reach full term can vary substantially between litters but will in most cases range between

114-117 days. In a retrospective study of more than 60.000 sow records, Vanderhaeghe and coworkers found a prevalence of 10% early deliveries (defined as <114 days) and that early birth coincided with a higher litter size and higher number of stillborn (Vanderhaeghe et al., 2011). As in humans, it is plausible that early delivery in pigs *per se* is a risk factor for mortality and morbidity. Likewise, it may be possible that piglets within a litter will display variation in degree of maturity at the time of birth. Provided that newborn pigs show varying degrees of maturity at the time of birth, this may account for some of the morbidity and mortality seen in pigs compared with any other mammal species. From this notion, there is a rationale to understand the pathophysiological mechanisms of prematurity in pigs, to identify ways to improve survival in the first days after birth.

To achieve a better understanding of the influence of prematurity, several studies were conducted with cesarean-derived newborn pigs. Strategies to improve survival have included respiratory support, controlled gut colonization, immunological support and nutritional interventions. To maximize the sensitivity toward these interventions, newborn piglets with a much higher degree of prematurity (i.e. born 10-12 days before expected term) than seen under normal circumstances were studied. In brief, pregnant sows were subjected to cesarean section on day 106-108 (Sangild et al., 2013). At this stage of pregnancy pigs require respiratory support, very controlled environmental conditions and careful nutrition, as neither the pulmonary-, circulatory-, thermoregulatory-, immunological-, or digestive system is fully developed. Under experimental conditions, these systems can be sufficiently supported to achieve a similar mortality rate as in pigs born at full term.

#### 4.2.2. Influence of prematurity on gut function and immunity

During fetal life, and particularly in the last trimester, the pig fetuses display swallowing movements, and ingest amniotic fluid. Amniotic fluid contains some of the same components as found in maternal colostrum and milk (e.g. IL-10, TGF- $\beta$ , EGF, IGF-1) (Siggers et al., 2013) and during the last trimester the fetuses swallow substantial amounts of amniotic fluid with marked

trophic effects on gut growth (Sangild et al., 2002; Trahair and Sangild, 2000). If fetal life is disrupted by premature birth, the gut trophic and maturational effects of amniotic fluid are not fully achieved and the intestine displays clear characteristics of prematurity. Postnatal feeding of preterm pigs with collected amniotic fluid has shown gut maturational effects (Siggers et al., 2013) albeit with varying degree of protection from gut disease (Ostergaard et al., 2014; Siggers et al., 2013). This may illustrate how the clinical effects of amniotic fluid depend on whether the intestine is sterile (during fetal life) or colonized with microbes after birth. From this notion, colostrum and milk may be more tailored to secure gut health with the concomitant influence of gut microbial colonization.

The prematurity characteristics of the gut include increased permeability (Hansen et al., 2016), reduced digestive and absorptive capacity (Buddington et al., 2008), increased sensitivity to necrotic and hemorrhagic changes, particularly in the colon and distal small intestine (Sangild et al., 2006). These pathological changes are collectively referred to as necrotizing enterocolitis (NEC). Although both etiology and pathogenesis remain enigmatic, there are three wellknown risk factors, i.e. prematurity, gut colonization and enteral feeding. The prematurity *per se* results in insufficient pulmonary surfactant production with poor expansion of the alveoli as a result. Together with a compromised circulatory function, this can result in poor saturation and poor tissue perfusion. The gut is sensitive to poor perfusion and it is plausible that ischemia and hypoxia are key etiologic factors for NEC. In this review we put more emphasis on the the other risk factor, i.e. gut colonization and its relation with enteral feeding (third risk factor).

#### 4.2.3. Prematurity and gut colonization

Following birth the gut is rapidly colonized. While the early colonizers and the succession of microbes depend on environmental bacteria, it is also partly dictated by the immaturity of the host (Cilieborg et al., 2011a). In human preterm neonates it is also influenced by extensive use of antibiotics, resulting in a dysbiotic and unstable gut microbiota. Although the advantages of antibiotics use outweigh the negative effects in preterm human infants, there is a need to

identify ways to stabilize the gut microbiota after antibiotics use. Similarly, antibiotics are commonly used for neonatal pigs under farming conditions to prevent or treat against pathogens. Using a combination of ampicillin, gentamicin and metronidazole, Jensen et al. (2014) showed that suppression of the early colonizers, allows the preterm gut to better adapt to postnatal life and that the protective effect was more pronounced if antibiotics were administered enterally relative to parenteral administration (Birck et al., 2016; Nguyen et al., 2016). Considering the dysbiosis seen in preterm infants, and considering frequent antibiotics use as a premise, it becomes relevant to use probiotics, prebiotics and synbiotics to support gut homeostasis after preterm birth (Johnson-Henry et al., 2016; Sawh et al., 2016). Use of probiotics in preterm human infants has been studied to some extent, yet the evidence for a positive and reproducible outcome is still weak. Whereas most studies do find positive effects of probiotics use against NEC and/or sepsis (Sawh et al., 2016), the effect can also be entirely neutral as indicated in the largest preterm infant probiotics experiment to date (Costeloe et al., 2016). Likewise, in preterm piglets both positive effects (Siggers et al., 2008) and negative effects (Cilieborg et al., 2011c) have been observed, indicating that details regarding choice of strain, timing, dosing and route of administration still need to be optimized to achieve a reproducible and positive clinical outcome.

The influence of gut dysbiosis after preterm birth is exacerbated if enteral nutrition is suboptimal. Following preterm birth, it is well known that mother's milk is more protective against NEC and sepsis than artificial formulas (Gupta and Paria, 2016). For pigs this is even more important as there is no transplacental transfer of immunoglobulins before birth, and the passive immunization therefore has to take place via colostrum ingestion immediately after birth (see also 2). Colostrum provides not only immunoglobulins, but also a range of other compounds including antibacterial, anti-inflammatory, immunoregulatory and growth stimulating factors, that support the gut in the transition to postnatal life outside the uterus. In preterm pigs, provision of colostrum is the most effective way to prevent NEC (Sangild et al., 2006) and the effect appears to be present also when using bovine colostrum (Bjornvad et al.,

2008). Although there are species-specific compounds in colostrum (e.g. porcine IgG), there also appears to be a protective effect of factors not specific to pigs. The gut protective effects of bovine colostrum in preterm pigs were studied in a number of studies (Andersen et al., 2016; Cilieborg et al., 2011b; Hansen et al., 2016; Sangild et al., 2006; Sty et al., 2016), and shown how it in most cases can prevent NEC-like lesions. Next, this creates an incentive for formulation of milk replacers that can mimic the effects of colostrum. Factors with immunomodulatory effects, e.g. osteopontin, gangliosides, sialic acid (Moller et al., 2011) or gut trophic effects e.g. GLP-2 and EGF (Benight et al., 2013; Sangild et al., 2006), have been tested in preterm pigs but in general the responses have been minor when tested using milk replacer as base diet. Among the nutritional interventions tested, replacing maltodextrin with lactose has shown most pronounced and reproducible effects (Buddington et al., 2008; Thymann et al., 2009a)(see also 1). It remains unclear whether this should be interpreted as protective effects of lactose or detrimental effects of maltodextrin. In pigs, the lactase enzyme is the most dominating carbohydrase after both preterm and term birth, which may in part explain the positive response to lactose relative to maltodextrin. In addition, lactose may serve other important functions directly in the mucosa. Blood samples collected directly from the portal vein under controlled jejunal infusions of lactose, showed that the derivatives of lactose, i.e. glucose and galactose, were not recaptured in a 1:1 ratio in blood although this is how they exist in the lactose molecule (Thymann et al., 2009b). Only half of the infused galactose was recaptured in the portal vein, indicating that it may be used for mucin synthesis or it may be a preferred substrate for mucosal or microbial metabolism.

#### 4.3. Nutritional modulation

##### 4.3.1. Sow's colostrum and milk

The intake of colostrum and milk is of crucial importance for the development of the GIT and immune system and therefore affects the survival and growth of the neonatal piglets (see sections above). Colostrum and milk are composed of macronutrients, but contain as well



immunoglobulins and immune cells, bioactive molecules such as hormones (e.g. insulin, neurotensin, bombesin, progesterone) and growth factors (e.g. IGF-I, EGF, TGF- $\beta$ ) and prebiotic and antimicrobial compounds. The latter two also playing a role on the establishment of the gut microbiota (Devillers and Lessard, 2007). The concentrations of the macronutrients undergo large changes during the lactation period. A fast drop in the protein concentration, mainly due to a decrease in immunoglobulins, is observed, while the concentration of the energetic molecules, i.e. lipids and lactose increase (Devillers and Lessard, 2007). Concerning porcine milk oligosaccharides, about 30 molecules have been identified, and changes in their composition also occur throughout lactation (Tao et al., 2010; Salcedo et al., 2016). For an extensive overview on the composition and importance of colostrum and milk, we refer to the paper of Devillers and Lessard (2007). In the following sections, we describe interventions through the maternal diet or by direct interventions to piglets in the pre-weaning period.

#### 4.3.2. Indirect intervention through maternal effects

Several studies have been investigating the effect of the supplementation of biological active compounds, such as prebiotics, or probiotics to sows and their effects on performance, immune development and the gut microbiome of their progeny. While very often effects on intestinal health or microbiota are observed, the performance of piglets until weaning remains sometimes unaffected (Leonard et al., 2012; Le Bourgnot et al., 2014).

The programming and modulation of maternal dietary changes will be discussed in the following sections, but the proof-of-principle has been nicely shown by a study on maternal antibiotic treatment modulating the progeny-pigs and its microbiota on a short- and long-term (Arnal et al., 2014). Indeed, maternal antibiotic treatment from 10 days before the estimated farrowing date until 21 days after farrowing, transiently modified both mother fecal and offspring ileal microbiota during the first weeks of life, without effects on offspring's microbiota on a long-term (169 days of age). The maternal antibiotic treatment transiently induced diverse temporal and

regional patterns of selective modifications in crypt depth (reduction), intestinal alkaline phosphatase activity and HSP70 protein production that suggest a lower or delayed response to bacteria especially in the ileum. In addition, site- and sometimes diet-specific long-term effects on key components of intestinal homeostasis (intestinal alkaline phosphatase, DPP-IV) were observed, without alterations on growth performance (Arnal et al., 2014).

#### 4.3.2.1. Effects on the microbiota

A nutritional modulation of the intestinal microbiota of sows affects bacterial community in the gastrointestinal tract of their suckling piglets, as contact with sow's faeces contributes to the microbial colonization in their offspring. This modulation has been shown to occur for probiotics (Macha et al., 2004; Baker et al., 2013; Starke et al., 2013) and for prebiotics (Paßlack et al., 2015). Modifications of microbial profiles were observed in suckling piglets although the altered profile did not completely mirror the quantitative composition in the sow suggesting modifications took place within the intestine of suckling piglets (Vahjen et al., 2007; Starke et al., 2013). Moreover, the influence diminished after weaning probably due to the natural bacterial development as piglets age (see also section 3), superseding the priming effect of a modified maternal microbiota (Starke et al., 2013). In their study, the authors observed that some sows reacted differently to the probiotic supplementation (*E. faecium* NCIMB), which led to the responder hypothesis that proposes an individual response to probiotic supplementation depending on the actual microbiota composition (Starke et al., 2013).

Dietary inulin supplemented to sows during the gestation and lactation period, increased the cell numbers of enterococci in sows' feces and in caecal digesta of the suckling piglets, showing the connection between the composition of the intestinal microbiota of mothers and their offspring (Bian et al. 2016). In line with this, Thompson et al. (2008) showed that cohoused piglets, raised by a sow milk replacer, developed very similar communities, indicating the direct environment and contact with other animals as important external factors affecting the development of bacterial communities (see also section 3).

#### 4.3.2.2. Effects on the immune system of the progeny

Supplementing sows with oligosaccharides such as short-chain fructooligosaccharides (scFOS), mannan-oligosaccharides (MOS) or a seaweed extract containing laminarin, a  $\beta$ - (1-3)/(1-6)glucan, might induce a nonspecific immune response responsible to increase colostral immunity (IgA, IgG or TGF $\beta$ ) (Czech et al., 2010; Leonard et al., 2010; 2012; Le Bourgot et al., 2014). The altered synthesis of key cytokines into mammary secretions might then influence the maturation and development of immune cells in offspring (Donnet-Hughes et al., 2000; Nguyen et al., 2007). This was observed in the study of Leonard et al. (2010) where gestating and lactating sows were supplemented with a seaweed extract (SWE) and piglets had a greater percentage of *E. coli* phagocytizing leukocytes and a decreased percentage of *E. coli* phagocytizing lymphocytes at weaning. Moreover the results after an *ex vivo* LPS challenge on ileal biopsies indicated that piglets from SWE supplemented sows had an enhanced immune function (Leonard et al., 2012). A later study on the supplementation of laminarin and/or fucoidan to sows showed that laminarin induced positive effects on intestinal architecture and health on day 8 postweaning and improved growth performance during the grower-finisher period (Heim et al., 2015). The authors proposed that the down-regulation of IL-6 gene expression may be attributed to the increased *Lactobacillus* spp. gene numbers in the colon of piglets and that the down-regulation of the pro-inflammatory cytokines may provide more nutrients available for growth. Alternatively, an increased secretion of pro-inflammatory cytokines (IFN $\gamma$ ) by Peyer's Patches (PP) and mesenteric lymph nodes cells, a higher proportion of activated T cells in ileal PP, and increased secretion of sIgA by PP cells were observed in suckling offspring of scFOS supplemented sows (Le Bourgot et al., 2014). The authors hypothesized that the colostral immunoglobulin and cytokine concentration induced by scFOS may modify the early process of commensal microbiota establishment in the intestine and of sIgA responses needed to maintain gut homeostasis.

Besides prebiotics, also conjugated linoleic acid or oils rich in omega-3 fatty acids provided to the sows during gestation and/or lactation have been shown to increase colostral immunoglobulins (Bontempo et al., 2004; Mitre et al., 2005; Corino et al., 2009), improve piglet body weight (Mitre et al., 2005; Corino et al., 2009), increase immune components (Fritsche et al., 1993; Bontempo et al., 2004; Mitre et al., 2005; Patterson et al., 2008; Corino et al., 2009) and reduce intestinal inflammation (Patterson et al., 2008).

#### 4.3.3. Direct interventions in piglets during the pre-weaning period

##### 4.3.3.1. Formula versus maternal milk

Current pig breeding uses hybrid sows with a high prolificacy (De Vos et al 2014), leading to the need of supplementing piglets with formula milk. As sow's milk contains bioactive compounds such as cytokines, hormones, immunoglobulins, stimulating and regulating the growth and maturation of the intestine (Devillers and Lessard, 2007), the composition of formula milk might also be enriched with several compounds to enhance gut development and modulate microbiota composition. The comparison between sow-reared and formula-fed piglets showed a comparable daily weight gain on day 28 of age, a greater absorptive area, deeper crypts, and higher maltase and sucrase activities in the small intestine compared to suckling piglets, after a delay in the functional maturation, reflected by a decreased lactase, dipeptidylpeptidase IV and sucrase activities on day 10 (De Vos et al., 2014). In the cecal contents of 21-d old piglets, *Prevotella* was the dominant genus within mother-fed samples and *Bacteroides* was most abundant within formula-fed samples (Poroyko et al., 2010). A metatranscriptomic analysis revealed increased transcripts for utilization of L-arabinose, a sugar known to accumulate in the distal intestine of formula-fed pigs due to the hydrolysis of plant-based non-starch polysaccharides additives (Schutte et al., 1992), and for utilization of the sugar alcohol mannitol in formula fed samples. Several differences were observed for transcripts encoding amino acid metabolism, and sow-fed samples had enriched sequences assigned to oxidative stress, which could be consistent with antioxidant properties of maternal milk (Poroyko et al., 2010). Other

studies support differential microbiota composition and metabolic activity between sow-reared and formula-fed piglets (Li et al., 2012; Wang et al., 2013).

#### 4.3.3.2. Milk composition

Prebiotics can be added to artificial milk to mimic the bioactive compounds present in maternal milk. The addition of scFOS and polydextrose to formula milk modulated microbial colonization and produced short-chain fatty acids pattern closer to that of sow-reared piglets, as seen by similar propionate and butyrate concentrations on d14 (Wang et al., 2013). In agreement with this modulation, Howard et al. (1995) observed that FOS was able to stimulate the bifidobacteria population in piglet's feces and Alizadeh et al. (2015) observed an increase in fecal lactobacilli and bifidobacteria in formula-fed piglets supplemented with galacto-oligosaccharides. Moreover, an increased cecal butyrate concentration was observed, which is known to prevent the colonization of various pathogens such as *E. coli*, to inhibit inflammation and to improve gut barrier function (Hinnebusch et al., 2003; Peng et al., 2009). The latter seemed to be stimulated by dietary GOS, given for 26 d (Alizadeh et al., 2015). Prebiotics in formula milk can furthermore improve intestinal architecture (Alizadeh et al., 2015) and enhance epithelial cell proliferation (Howard et al., 1995). In addition, prefeeding of inulin reversed the inhibitory effects of a later supplemented laxative dose of lactulose on cecal cell proliferation and diarrhea (Kien et al., 2004).

Besides prebiotics, high protein (HP) concentration in formula milk has been shown to affect microbiota proliferation on d7 (Chatelais et al., 2011), without changing the microbiota composition on d28, increase intestinal transcellular permeability (Chatelais et al., 2011; Boudry et al., 2013) and modify immune development (Chatelais et al., 2011; Boudry et al., 2013). Later in life, HP formula diet increased colonic permeability (Boudry et al., 2013) and affected the ileal and colonic response to inflammatory mediators in a gender specific way (Chatelais et al., 2011; Boudry et al., 2013), probably through microbial and hormonal factors (Boudry et al., 2013).

## 714 Conclusion

715 In conclusion, the GIT of neonatal piglets undergoes important maturation processes and  
716 establishes its ecosystem, both being far from complete by the time of weaning. Even more,  
717 these processes will be disturbed during the weaning period, and strategies should focus to  
718 minimize the impact of this abrupt weaning on the GIT and its microbial community.  
719 Furthermore, preterm birth in pigs, even a few days before the expected term date, and reflected  
720 by a low birth weight is a major risk factor for gut dysbiosis and mucosal dysfunction. Dietary  
721 strategies such as the use of pre- and probiotics applied during the perinatal period should try to  
722 stabilize the gut microbiota and stimulate the innate immunity. It becomes more and more  
723 evident that long-term effects are provoked by early-life interventions on microbiota  
724 composition and gut homeostasis. Thus, while most of the studies on nutritional modulation  
725 report mainly on a short-term, it is expected that future research will also reveal strategies to  
726 improve gut homeostasis at the physiological, immunological and microbial level on the long-  
727 term.

728

## 729 Acknowledgments

730 The authors would like to acknowledge the COST Action FA1401 “European network on the  
731 factors affecting the gastro-intestinal microbial balance and the impact on the health status of  
732 pigs (PiGutNet)” for instigating this review.

## References

- Alizadeh, A., Akbari, P., Difilippo, E., Schols, H. A., Ulfman, L.H., Schoterman, M.H.C., Garssen, J., Fink-Gremmels, J., Braber, S., 2015. The piglet as a model for studying dietary components in infant diets: effects of galacto-oligosaccharides on intestinal functions. *Br. J. Nutr.* 115, 605-618. doi:10.1017/S0007114515004997.
- Amdi, C., Krogh, U., Flummer, C., Oksbjerg, N., Hansen, C.F., Theil, P.K., 2013. Intrauterine growth restricted piglets defined by their head shape ingest insufficient amounts of colostrum. *J. Anim. Sci.* 91, 5605-5613.
- Andersen, A.D., Sangild, P.T., Munch, S.L., van der Beek, E.M., Renes, I.B., Ginneken, C., Greisen, G.O., Thymann, T., 2016. Delayed growth, motor function and learning in preterm pigs during early postnatal life. *Am. J. Physiol Regul. Integr. Comp Physiol* 310, R481-R492.
- Arnal, M.E., Zhang, J., Messori, S., Bosi, P., Smidt, H., Lallès, J.P., 2014. Early changes in microbial colonization selectively modulate intestinal enzymes, but not inducible heat shock proteins in young adult swine. *PLoS One* 9. doi:10.1371/journal.pone.0087967
- Ashworth, C.J., Finch, A.M., Page, K.R., Nwagwu, M.O., McArdle, H.J., 2001. Causes and consequences of fetal growth retardation in pigs. *Control of Pig Production VI* 58, 233-246.
- Baintner, K., 1986. Intestinal absorption of macromolecules and immune transmission from mother to young. CRC Press (Boca Raton, Fl.), 1-216.
- Bach, T.A., Carey, H. V., 1994. Developmental-Changes in Neurally-Mediated Ion-Transport in Piglet Distal Colon. *Pediatr. Res.* 36, 144-151.
- Bailey, M., Haverson, K., Inman, C., Harris, C., Jones, P., Corfield, G., Miller, B., and Stokes, C., 2005. The development of the mucosal immune system pre- and post-weaning: balancing regulatory and effector function. *Proc. Nutr. Soc.* 64, 451-457.
- Baker, A.A., Davis, E., Spencer, J.D., Moser, R., Rehberger, T., 2013. The effect of a *Bacillus*-based direct-fed microbial supplemented to sows on the gastrointestinal microbiota of their neonatal piglets. *J. Anim. Sci.* 91, 3390-3399. doi:10.2527/jas.2012-5821

760 Barman, N.N., Bianchi, A.T.J., Zwart, R.J., Pabst, R., and Rothkotter, H.J., 1997. Jejunal and ileal  
761 Peyer's patches in pigs differ in their postnatal development. *Anat. Embryol., Berl.* 195,  
762 41–50.

763 Beaulieu, A.D., Aalhus, J.L., Williams, N.H., Patience, J.F., 2010. Impact of piglet birth weight, birth  
764 order and litter size on subsequent growth performance, carcass quality, muscle  
765 composition and eating quality of pork. *J. Anim. Sci.* 88, 2767-2778.

766 Bee, G., 2007. Birth weight of litters as a source of variation in postnatal growth, and carcass and  
767 meat quality. *Adv. Pork Product.* 18, 191-196.

768 Benight, N.M., Stoll, B., Olutoye, O.O., Holst, J.J., Burrin, D.G., 2013. GLP-2 delays but does not  
769 prevent the onset of necrotizing enterocolitis in preterm pigs. *J. Pediatr. Gastroenterol.*  
770 *Nutr.* 56, 623-630.

771 Bian, G., Ma, S., Zhu, Z., Su, Y., Zoetendal, E.G., Mackie, R., Liu, J., Mu, C., Huang, R., Smidt, H., Zhu,  
772 W., 2016. Age, introduction of solid feed and weaning are more important determinants  
773 of gut bacterial succession in piglets than breed and nursing mother as revealed by a  
774 reciprocal cross-fostering model. *Environ. Microbiol.* 18, 1566–1577. doi:10.1111/1462-  
775 2920.13272

776 Bianchi, A., Zwart, R., Jeurissen, S., and Moonenleusen, H., 1992. Development of the B-Cell and T-  
777 Cell Compartments in Porcine Lymphoid Organs from Birth to Adult Life - an  
778 Immunohistological Approach. *Vet. Immunol. Immunopathol.* 33, 201–221.

779 Biernat, M., Wolinski, J., Godlewski, M.M., Motyl, T., Morisset, J., Zabielski, R., 2003. Apoptosis in  
780 the gut of neonatal piglets. *Proceedings of the 9th International Symposium on Digestive*  
781 *Physiology in Pigs.* University of Alberta, Edmonton, Canada, 46-48.

782 Birck, M.M., Nguyen, D.N., Cilieborg, M.S., Kamal, S.S., Nielsen, D.S., Damborg, P., Olsen J.E.,  
783 Lauridsen, C., Sangild, P.T., Thymann, T., 2016. Enteral but not parenteral antibiotics  
784 enhance gut function and prevent necrotizing enterocolitis in formula-fed newborn  
785 preterm pigs. *Am. J. Physiol. Gastrointest. Liver Physiol.* 310, G323-G333.



786 Bjornvad, C.R., Thymann, T., Deutz, N.E., Burrin, D.G., Jensen, S.K., Jensen, B.B., Molbak, L., Boye,  
 787 M., Larsson, L.I., Schmidt, M., Michaelsen, K.F., Sangild, P.T., 2008. Enteral feeding induces  
 788 diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing  
 789 enterocolitis in preterm pigs on parenteral nutrition. *Am. J. Physiol. Gastrointest. Liver*  
 790 *Physiol.* 295, G1092-G1103.

791 Bontempo, V., Sciannimanico, D., Pastorelli, G., Rossi, R., Rosi, F., Corino, C., 2004. in *Lactating*  
 792 *Sows and Piglets* 1, 2. *J. Nutr.* 817–824.

793 Boudry, G., Morise, A., Seve, B., Le Huërou-Luron, I., 2011. Effect of milk formula protein content  
 794 on intestinal barrier function in a porcine model of LBW neonates. *Ped. Res.* 69, 4-9.

795 Boudry, G., Jamin, A., Chatelais, L., Gras-Le Guen, C., Michel, C., Le Huërou-Luron, I., 2013. Dietary  
 796 protein excess during neonatal life alters colonic microbiota and mucosal response to  
 797 inflammatory mediators later in life in female pigs. *J. Nutr.* 143, 1225–1232.

798 Bourgot, C. Le, Ferret-Bernard, S., Normand, L. Le, Savary, G., Menendez-Aparicio, E., Blat, S.,  
 799 Appert-Bossard, E., Respondek, F., Le Huërou-Luron, I., 2014. Maternal short-chain  
 800 fructooligosaccharide supplementation influences intestinal immune system maturation  
 801 in piglets. *PLoS One* 9, 1–12. doi:10.1371/journal.pone.0107508

802 Brambell, F.W.R., 1970. *The Transmission of passive immunity from mother to young.* North-  
 803 *Holland Publ. Company (Amsterdam)*, 1-385.

804 Braude, R., Newport, M.J., Porter, J.W.G., 1970. Artificial Rearing of Pigs .2. Time course of milk  
 805 protein digestion and proteolytic enzyme secretion in 28-day-old pig. *Br. J. Nutr.* 24, 827-  
 806 842.

807 Buddington, R.K., Bering, S.B., Thymann, T., Sangild, P.T., 2008. Aldohexose malabsorption in  
 808 preterm pigs is directly related to the severity of necrotizing enterocolitis. *Pediatr. Res.*  
 809 63, 382-387.

810 Buddington, R.K., Elnif, J., Puchal-Gardiner, A.A., Sangild, P.T., 2001. Intestinal apical amino acid  
 811 absorption during development of the pig. *Am. J. Physiol. - Reg. Integr. Comp. Physiol.*,  
 812 280, R241-R247.

813 Buddington, R.K., Malo, C., 1996. Intestinal brush-border membrane enzyme activities and  
814 transport functions during prenatal development of pigs. *J. Pediatr. Gastroenterol. Nutr.*  
815 23, 51-64.

816 Buddington, R.K., Sangild, P.T., 2011. Companion animals symposium: Development of the  
817 mammalian gastrointestinal tract, the resident microbiota, and the role of diet in early  
818 life. *J. Anim. Sci.* 89, 1506-1519.

819 Burkey, T.E., Skjolaas, K.A., Minton, J.E., Board-invited review: porcine mucosal immunity of the  
820 gastrointestinal tract. *J. Anim. Sci.*, 87, 1493-1501.

821 Butler, J.E., Santiago-Mateo, K., Wertz, N., Sun, X., Sinkora, M., and Francis, D.L., 2016. Antibody  
822 repertoire development in fetal and neonatal piglets. XXIV. Hypothesis: The ileal Peyer  
823 patches, IPP are the major source of primary, undiversified IgA antibodies in newborn  
824 piglets. *Dev. Comp. Immunol.* 65, 340-351.

825 Carver, J.D., Barness, L.A., 1996. Trophic factors for the gastrointestinal tract. *Clin. Perinatol.* 23,  
826 265-285.

827 Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A., Whitmoyer, R. E., 1988. Effect of age, weaning  
828 and postweaning diet on small intestinal growth and jejunal morphology in young swine.  
829 *J. Anim. Sci.* 66, 574-584.

830 Chatelais, L., Jamin, A., Guen, C.G. Le, Lallès, J.P., le Huërou-Luron, I., Boudry, G., 2011. The level of  
831 protein in milk formula modifies ileal sensitivity to LPS later in life in a piglet model.  
832 *PLoS One* 6, 1-10. doi:10.1371/journal.pone.0019594

833 Che, L., Thymann, T., Bering, S.B., Le Huërou-Luron, I., D'Inca, R., Zhang, K., Sangild, P.T., 2010.  
834 IUGR does not predispose to necrotizing enterocolitis or compromise postnatal intestinal  
835 adaptation in preterm pigs. *Pediatr. Res.* 67, 54-59.

836 Cilieborg, M.S., Boye, M., Molbak, L., Thymann, T., Sangild, P.T., 2011a. Preterm birth and  
837 necrotizing enterocolitis alter gut colonization in pigs. *Pediatr. Res.* 69, 10-16.

838 Cilieborg, M.S., Boye, M., Thymann, T., Jensen, B.B., Sangild, P.T., 2011b. Diet-dependent effects of  
 839 minimal enteral nutrition on intestinal function and necrotizing enterocolitis in preterm  
 840 pigs. *J. Parenter. Enteral Nutr.* 35, 32-42.

841 Cilieborg, M.S., Thymann, T., Siggers, R., Boye, M., Bering, S.B., Jensen, B.B., Sangild, P.T., 2011c.  
 842 The incidence of necrotizing enterocolitis is increased following probiotic administration  
 843 to preterm pigs. *J. Nutr.* 141, 223-230.

844 Collado, M.C., Cernada, M., Bauerl, C., Vento, M., and Perez-Martinez, G., 2012. Microbial ecology  
 845 and host-microbiota interactions during early life stages. *Gut Microbes* 3, 352–365.

846 Cooper, J.E., 1975. The use of the pig as an animal model to study problems associated with low  
 847 birthweight. *Lab. Anim.* 9, 329-336.

848 Corino, C., Pastorelli, G., Rosi, F., Bontempo, V., Rossi, R., 2009. Effect of dietary conjugated  
 849 linoleic acid supplementation in sows on performance and immunoglobulin  
 850 concentration in piglets. *J Anim Sci* 87, 2299–2305. doi:10.2527/jas.2008-1232.

851 Costeloe, K., Bowler, U., Brocklehurst, P., Hardy, P., Heal, P., Juszczak, E., King, A., Panton, N.,  
 852 Stacey, F., Whiley, A., Wilks, M., Millar, M.R., 2016. A randomised controlled trial of the  
 853 probiotic *Bifidobacterium breve* BBG-001 in preterm babies to prevent sepsis,  
 854 necrotising enterocolitis and death: the Probiotics in Preterm infantS (PiPS) trial. *Health*  
 855 *Technol. Assess.* 20, 1-194.

856 Cranwell, P. D., 1985. The development of acid and pepsin (EC 3.4.23.1) secretory capacity in the  
 857 pig: the effects of age and weaning. *Br. J. Nutr.* 54, 305-320.

858 Cranwell, P.D., 1995. Development of the neonatal gut and enzyme systems, in: Varley M. A. (ed.),  
 859 The Neonatal Pig, Development and Survival. CAB international, Wallingford, UK. pp. 99-  
 860 154.

861 Cremaschi, D., Ferguson, D.R., Henin, S., James, P.S., Meyer, G., Smith, M.W., 1979. Postnatal-  
 862 Development of Amiloride Sensitive Sodium-Transport in Pig Distal Colon. *J. Physiol.*  
 863 292, 481-494.

864 Czech, A., Grela, E.R., Mokrzycka, A., Pejsak, Z., 2010. Efficacy of mannanoligosaccharides additive  
865 to sows diets on colostrum, blood immunoglobulin content and production parameters  
866 of piglets. *Pol. J. Vet. Sci.* 13, 525–31.

867 Davies, S., 2010. Further reading 3, 3943–3947.  
868 doi:<http://dx.doi.org/10.1533/9781782420569.183>

869 Decuypere, J.A., Dendooven, R.M., Henderickx, H.K., 1986. Stomach emptying of milk diets in pigs.  
870 A mathematical model allowing description and comparison of the emptying pattern.  
871 *Arch. Tierernahr* 36, 679-696.

872 Dethlefsen, L., McFall-Ngai, M., Relman, D.A., An ecological and evolutionary perspective on  
873 human-microbe mutualism and disease. *Nature*, 449, 811-818.

874 De Vos, M., Che, L., Huygelen, V., Willemen, S.A., Michiels, J., Van Cruchten, S., Van Ginneken, C.,  
875 2014. Nutritional interventions to prevent and rear low-birthweight piglets. *J. Anim.*  
876 *Physiol. Anim. Nutr.* 98, 609-619.

877 De Vos, M., Huygelen, V., Willemen, S., Fransen, E., Casteleyn, C., Van Cruchten, S., Michiels, J., Van  
878 Ginneken, C., 2014. Artificial rearing of piglets: Effects on small intestinal morphology  
879 and digestion capacity. *Livest. Sci.* 159, 165–173. doi:10.1016/j.livsci.2013.11.012

880 Devillers, N., Lessard, M., 2007. New concepts related to the importance of colostrum for modern  
881 pig production. Proceedings from 43rd Eastern Nutrition Conference of the Animal  
882 Nutrition Association of Canada, Quebec City, Quebec, Canada.

883 D'Inca, R., Gras-Le Guen, C., Che, L., Sangild, P.T., Le Huërou-Luron, I., 2011. Intrauterine growth  
884 restriction delays feeding-induced gut adaptation in term newborn pigs. *Neonatal.* 99,  
885 208-216.

886 D'Inca, R., Kloareg, M., Gras-Le Guen, C., Le Huërou-Luron, I., 2010. Intrauterine growth  
887 restriction modifies the developmental pattern of intestinal structure, transcriptomic  
888 profile and bacterial colonization in neonatal piglets. *J. Nutr.* 140, 925-931.

889 Donnet-Hughes, A., Duc, N., Serrant, P., Vidal, K., Schiffrin, E.J., 2000. Bioactive molecules in milk  
890 and their role in health and disease: the role of transforming growth factor-beta.  
891 Immunol Cell Biol 78, 74–79. doi:icb882 [pii]

892 Ekström, G.M., Weström, B.R., Telemo, E., Karlsson, B.W., 1988. The uptake of fluorescein-  
893 conjugated dextran 70.000 by the small intestinal epithelium of the young rat and pig in  
894 relation to macromolecular transmission into blood. J. Develop. Physiol. 10, 227-233.

895 Ewing W.N., Tucker, L.A., 2008. The living gut. Nottingham University press. 2nd ed.

896 Ferenc, K., Pietrzak, P., Godlewski, M.M., Piwowarski, J., Kilianczyk, R., Guilloteau, P., Zabielski, R.,  
897 2014. Intrauterine growth retarded piglet as a model for humans - studies on the  
898 perinatal development of gut structure and function. Reprod. Biol. 14, 51-60.

899 Fritsche, K.L., Alexander, D.W., Cassity, N.A., Huang, S.C., 1993. Maternally-supplied fish oil alters  
900 piglet immune cell fatty acid profile and eicosanoid production. Lipids 28, 677–682.  
901 doi:10.1007/BF02535986

902 Georgieva, R., Gerov, K., 1975a. The morphological and functional differentiation of the  
903 alimentary canal of the pig during ontogeny. I. Development and differentiation of the  
904 fundic portion of the stomach. Anat. Anz. 137, 12-15.

905 Georgieva, R., Gerov, K., 1975b. The morphological and functional differentiation of the  
906 alimentary canal of the pig during ontogeny. II. Development and differentiation of the  
907 jejunum. Anat. Anz. 137, 16-20.

908 Godlewski, M.M., Hallay, N., Bierla, J.B., Zabielski, R., 2007. Molecular mechanism of programmed  
909 cell death in the gut epithelium of neonatal piglets. J. Physiol. Pharmacol. 58, 97-113.

910 Godlewski, M.M., Slupecka, M., Wolinski, J., Skrzypek, T., Skrzypek, H., Motyl, T., Zabielski, R.,  
911 2005. Into the unknown- The death pathways in the neonatal gut epithelium. J. Physiol.  
912 Pharmacol. 56 (supp. 3), 7-24.

913 Gondret, F., Lefaucheur, L., Louveau, I., Lebret, B., Pichodo, X., Le Cozler, Y., 2002. Influence of  
914 piglet birth weight on postnatal growth performance, tissue lipogenic capacity and  
915 muscle histological traits at market weight. Prev. Vet. Med. 56, 119-127.

916 Grześkowiak, Ł., Zentek, J., Vahjen, W.. 2016, Determination of the extent of *Clostridium difficile*  
 917 colonisation and toxin accumulation in sows and neonatal piglets. *Anaerobe* 40, 5-9.

918 Gupta, A., Paria, A. 2016. Etiology and medical management of NEC. *Early Hum. Dev.* 97, 17-23.

919 Hall, P. A., Coates, P.J., Ansari, B., Hopwood, D., 1994. Regulation of Cell Number in the  
 920 Mammalian Gastrointestinal-Tract - the Importance of Apoptosis. *J. Cell Sci.* 107, 3569-  
 921 3577.

922 Hampson, D.J., Kidder, D.E., 1986. Influence of creep feeding and weaning on brush-border  
 923 enzyme-activities in the piglet small-intestine. *Res. Vet. Sci.* 40, 24-31.

924 Hansen, M.B., 2003. The enteric nervous system II: Gastrointestinal functions. *Pharmacol.*  
 925 *Toxicol.* 92, 249-257.

926 Hansen, C.F., Thymann, T., Andersen, A.D., Holst, J.J., Hartmann, B., Hilsted, L., Langhorn, L.,  
 927 Jelsing, J., Sangild, P.T., 2016. Rapid gut growth but persistent delay in digestive function  
 928 in the postnatal period of preterm pigs. *Am. J. Physiol Gastrointest. Liver Physiol* 310,  
 929 G550-G560.

930 Heim, G., Sweeney, T., O'Shea, C.J., Doyle, D.N., O'Doherty, J. V., 2015. Effect of maternal dietary  
 931 supplementation of laminarin and fucoidan, independently or in combination, on pig  
 932 growth performance and aspects of intestinal health. *Anim. Feed Sci. Technol.* 204, 28-  
 933 41. doi:10.1016/j.anifeedsci.2015.02.007

934 Henning, S.J., Rubin, D.C., Shulman, R.J. 1994. Ontogeny of the intestinal mucosa. in: Johnson, L. R.  
 935 (ed.), *Physiology of the gastrointestinal tract*. 3rd ed., Raven Press, New York, pp 571-  
 936 610.

937 Hinnebusch, B.F., Henderson, J.W., Siddique, A., Malo, M.S., Zhang, W, Abedrapo, M.A, Hodin, R.A.,  
 938 2003. Transcriptional activation of the enterocyte differentiation marker intestinal  
 939 alkaline phosphatase is associated with changes in the acetylation state of histone H3 at  
 940 a specific site within its promoter region in vitro. *J. Gastrointest. Surg.* 7, 237-45.

941 Howard M.D., Gordon, D.T., Pace, L.W., Garleb, K.A., Kerley, M.S., 1995. Effects of dietary  
942 supplementation with fructooligosaccharides on colonic microbiota populations and  
943 epithelial cell proliferation in neonatal pigs. *J. Pediatr. Gastroenterol. Nutr.* 21, 297-303.

944 Huygelen, V., De Vos, M., Willemen, S., Fransen, E., Casteleyn, C., Van Cruchten, S., Van Ginneken,  
945 C., 2014. Age-related differences in mucosal barrier function and morphology of the  
946 small intestine in low and normal birth weight piglets. *J. Anim. Sci.* 92, 3398-3406.

947 Huygelen, V., De Vos, M., Prims, S., Vergauwen, H., Fransen, E., Casteleyn, C., Van Cruchten, S., Van  
948 Ginneken, C., 2015. Birth weight has no influence on the morphology, digestive capacity  
949 and motility of the small intestine in suckling pigs. *Livest. Sci.* 182, 129-136.

950 Inman, C.F., Singha, S., Lewis, M., Bradley, B., Stokes, C., and Bailey, M., 2010a. Dendritic cells  
951 interact with CD4 T cells in intestinal mucosa. *J. Leukoc. Biol.* 88, 571-578.

952 Inman, C.F., Haverson, K., Konstantinov, S.R., Jones, P.H., Harris, C., Smidt, H., Miller, B., Bailey, M.,  
953 and Stokes, C., 2010b. Rearing environment affects development of the immune system  
954 in neonates. *Clin. Exp. Immunol.* 160, 431-439.

955 Inman, C.F., Laycock, G.M., Mitchard, L., Harley, R., Warwick, J., Burt, R., van Diemen, P.M.,  
956 Stevens, M., and Bailey, M., 2012. Neonatal Colonisation Expands a Specific Intestinal  
957 Antigen-Presenting Cell Subset Prior to CD4 T-Cell Expansion, without Altering T-Cell  
958 Repertoire. *PLoS One* 7.

959 Israel, E.J., Simister, N., Freiberg, E., Caplan, A., Walker, W.A., 1993. Immunoglobulin G binding  
960 sites on the human foetal intestine: a possible mechanism for the passive transfer of  
961 immunity from mother to infant. *Immunol.* 79, 77-81.

962 Jakobsson, I., Lindberg, T., Lothe, L., Axelsson, I., Benediktsson, B., 1986. Human a-lactalbumin as  
963 a marker of macromolecular absorption. *Gut.* 27, 1029-1034.

964 Jensen, A.R., Elnif, J., Burrin, D.G., Sangild, P.R., 2001. Development of intestinal immunoglobulin  
965 absorption and enzyme activities in neonatal pigs is diet dependent. *J. Nutr.* 131, 3259-  
966 3265.

967 Jensen, M.L., Thymann, T., Cilieborg, M.S., Lykke, M., Molbak, L., Jensen, B.B., Schmidt, M., Kelly, D.,  
 968 Mulder, I., Burrin, D.G., Sangild, P.T., 2014. Antibiotics modulate intestinal immunity and  
 969 prevent necrotizing enterocolitis in preterm neonatal piglets. *Am. J. Physiol Gastrointest.*  
 970 *Liver Physiol.* 306, G59-G71.

971 Johnson-Henry, K.C., Abrahamsson, T.R., Wu, R.Y., Sherman, P.M., 2016. Probiotics, prebiotics,  
 972 and synbiotics for the prevention of necrotizing enterocolitis. *Adv. Nutr.* 7, 928-937.

973 Jones, E.A., Waldmann, T.A., 1971. The mechanism of intestinal uptake and transcellular  
 974 transport of IgG in the neonatal rat. *J. Clin. Invest.* 31, 2916-2927.

975 Kidder, D.E., Manners, M.J., 1968. Passage of food in young suckling pig. *Proc. Nutr. Soc.* 27, A46-  
 976 A47.

977 Kim, H.B., Borewicz, K., White, B.A., Singer, R.S., Sreevatsan, S., Tu, Z.J., Isaacson, R.E., 2011,  
 978 Longitudinal investigation of the age-related bacterial diversity in the feces of  
 979 commercial pigs. *Vet. Microbiol.*, 153, 124-133.

980 Klein, R.M., 1989. Small intestinal cell proliferation during development. in: Lebenthal, E. (ed.),  
 981 *Human Gastrointestinal Development*, Raven Press, New York, pp 367–394.

982 Kubasova, T., Davidova-Gerzova, L., Merlot, E., Medvecky, M., Polansky, O., Gardan-Salmon, D.,  
 983 Quesnel, H., Rychlik, I., 2017. Housing Systems Influence Gut Microbiota Composition of  
 984 Sows but Not of Their Piglets. *PLoS One*, 12, e0170051.

985 Koenig, J.E., Spor, A., Scalfone, N., Fricker, A.D., Stombaugh, J., Knight, R., Angenent, L.T., Ley, R.E.,  
 986 2011, Succession of microbial consortia in the developing infant gut microbiome. *PNAS*,  
 987 108, 4578-4585.

988 Konstantinov, S.R., Favier, C.F., Zhu, W.Y., Williams, B.A., Klüß, J., Souffrant, W.-B., de Vos, W.M.,  
 989 Akkermans, A.D.L., Smidt, H., 2004. Microbial diversity studies of the porcine  
 990 gastrointestinal ecosystem during weaning transition. *Anim. Res.* 53, 317–24

991 Lallès J.-P., Boudry, G., Favier, C., Le Floc'h, N., Luron, I., Montagne, L., Oswald, I., Pié, S., Piel, C.,  
 992 Sève, B., 2004. Gut function and dysfunction in young pigs: physiology *Anim. Res.* 53,  
 993 301–16



994 Lallès, J.-P., Bosi, P., Smidt, H., Stokes, C.R., 2007. Nutritional management of gut health in pigs  
995 around weaning. *Proc. Nutr. Soc.* 66, 260–268. doi:10.1017/S0029665107005484

996 Lamendella, R., Domingo, J.W.S., Ghosh, S., Martinson, J., Oerther D.B., 2011, Comparative fecal  
997 metagenomics unveils unique functional capacity of the swine gut, *BMC Microbiol.*, 11,  
998 103.

999 Le Huërou-Luron, I., 2002. Production and gene expression of brush border dissaccharidases  
1000 and peptidases during development in pigs and calves. In: *Biology of the intestine in*  
1001 *growing animals*. Ed. Zabielski, R., Gregory, P.C., Weström B., Elsevier Science  
1002 Amsterdam, p491-513.

1003 Lecce, J.G., 1973. Effect of Dietary Regimen on Cessation of Uptake of Macromolecules by piglet  
1004 intestinal epithelium (closure) and transport to blood. *J. Nutr.* 103, 751-756.

1005 Leonard, S.G., Sweeney, T., Bahar, B., O'Doherty, J.V., 2012. Effect of maternal seaweed extract  
1006 supplementation on suckling piglet growth, humoral immunity, selected microflora, and  
1007 immune response after an ex vivo lipopolysaccharide challenge. *J. Anim. Sci.* 90, 505–  
1008 514. doi:10.2527/jas.2010-3243

1009 Lewis, M.C., Inman, C.F., Patel, D., Schmidt, B., Mulder, I., Miller, B., Gill, B.P., Pluske, J., Kelly, D.,  
1010 Stokes, C.R., et al., 2012. Direct experimental evidence that early-life farm environment  
1011 influences regulation of immune responses. *Pediatr. Allergy Immunol.* 23, 265–269.

1012 Ley, R.E., Peterson, D.A., Gordon, J.I., 2006, Ecological and evolutionary forces shaping microbial  
1013 diversity in the human intestine. *Cell.* 124, 837-848.

1014 Looft T., Allen, H.K., Cantarel, B.L., Levine, U.Y., Bayles, D.O., Alt, D.P., Henrissat, B., Stanton, T.B.,  
1015 2014, Bacteria, phages and pigs: the effects of in-feed antibiotics on the microbiome at  
1016 different gut locations. *ISME J.*, 8, 1566-1576.

1017 Low, A.G., 1990. Nutritional regulation of gastric secretion, digestion and emptying. *Nutr. Res.*  
1018 Rev. 3, 229-252.

1019 Macha, M., Taras, D., Vahjen, W., Arini, A., Simon, O., 2004. Specific enumeration of the probiotic  
1020 strain *Enterococcus faecium* NCIMB 10415 in the intestinal tract and in faeces of piglets  
1021 and sows. *Arch. Anim. Nutr.* 58, 443–452. doi:10.1080/00039420400020058

1022 Makala, L.H.C., Kamada, T., Nishikawa, Y., Nagasawa, H., Igarashi, I., Fujisaki, K., Suzuki, N.,  
1023 Mikami, T., Haverson, K., Bailey, M., et al., 2000. Ontogeny of pig discrete Peyer's patches:  
1024 Distribution and morphometric analysis. *Pathobiology* 68, 275–282.

1025 McPherson, R.L., Ji, F., Wu, G., Blanton, J.R., Kim, S.W., 2004. Growth and compositional changes of  
1026 fetal tissues in pigs. *J. Anim. Sci.* 82, 2534-2540.

1027 Merrifield, C.A., Lewis, M., Berger, B., Cloarec, O., Heinzmann, S.S., Charton, F., Krause, L., Levin,  
1028 N.S., Duncker, S., Mercenier, A., Holmes, E., Bailey, M., Nicholson, J.K., 2015. Neonatal  
1029 environment exert a sustained influence on the development of the intestinal microbiota  
1030 and metabolic phenotype. *ISME J.* 10, 145-157.

1031 Miller, B.G., James, P.S., Smith, M.W., Bourne, F.J., 1986. Effect of weaning on the capacity of pig  
1032 intestinal villi to digest and absorb nutrients. *J. Agric. Sci.* 107, 579-589.

1033 Miller, M.S., Galligan, J.J., Burks, T.F., 1981. Accurate measurement of intestinal transit in the rat.  
1034 *J. Pharmacol. Methods* 6, 211-217.

1035 Mickiewicz, M., Zabielski, R., Grenier, B., Le Normand, L., Savary, G., Holst, J J., Oswald, I.P.,  
1036 Metges, C.C., Guilloteau, P., 2012. Structural and functional development of small  
1037 intestine in intrauterine growth retarded porcine offspring born to gilts fed diets with  
1038 differing protein ratios throughout pregnancy. *J. Physiol. Pharmacol.* 63, 225-239.

1039 Mitre, R., Etienne, M., Martinais, S., Salmon, H., Allaume, P., Legrand, P., Legrand, A.B., 2005.  
1040 Humoral defence improvement and haematopoiesis stimulation in sows and offspring by  
1041 oral supply of shark-liver oil to mothers during gestation and lactation. *Br. J. Nutr.* 94,  
1042 753–62. doi:10.1079/BJN20051569

1043 Moller, H.K., Thymann, T., Fink, L.N., Frokiaer, H., Kvistgaard, A.S., Sangild, P.T., 2011. Bovine  
1044 colostrum is superior to enriched formulas in stimulating intestinal function and  
1045 necrotising enterocolitis resistance in preterm pigs. *Br. J. Nutr.* 105, 44-53.

1046 Montedonico, S., Paran, T.S., Pirker, M., Rolle, U., Puri, P., 2006. Developmental changes in  
 1047 submucosal nitrergic neurons in the porcine distal colon. *J. Pediatr. Surg.* 41, 1029-1035.  
 1048 Moughan, P.J., Cranwell, P.D., Smith, W.C., 1991. An evaluation with piglets of bovine milk,  
 1049 hydrolyzed bovine milk, and isolated soybean proteins included in infant milk formulas.  
 1050 II. Stomach-emptying rate and the postprandial change in gastric pH and milk-clotting  
 1051 enzyme activity. *J. Pediatr. Gastroenterol. Nutr.* 12, 253-259.  
 1052 Mulder, I.E., Schmidt, B., Stokes, C.R., Lewis, M., Bailey, M., Aminov, R.I., Prosser, J.I., Gill, B.P.,  
 1053 Pluske, J.R., Mayer, C.-D., et al., 2009. Environmentally-acquired bacteria influence  
 1054 microbial diversity and natural innate immune responses at gut surfaces. *Bmc Biol.* 7.  
 1055 Mulder, I.E., Schmidt, B., Lewis, M., Delday, M., Stokes, C.R., Bailey, M., Aminov, R.I., Gill, B.P.,  
 1056 Pluske, J.R., Mayer, C.-D., et al., 2011. Restricting Microbial Exposure in Early Life Negates  
 1057 the Immune Benefits Associated with Gut Colonization in Environments of High  
 1058 Microbial Diversity. *Plos One* 6, e28279.  
 1059 Nguyen, D.N., Fuglsang, E., Jiang, P., Birck, M.M., Pan, X., Kamal, S.B., Pors, S.E., Gammelgaard, P.L.,  
 1060 Nielsen, D.S., Thymann, T., Levy, O., Frokiaer, H., Sangild, P.T., 2016. Oral antibiotics  
 1061 increase blood neutrophil maturation and reduce bacteremia and necrotizing  
 1062 enterocolitis in the immediate postnatal period of preterm pigs. *Innate. Immun.* 22:51-  
 1063 62.  
 1064 Nguyen, T.V., Yuan, L., Azevedo, M.S.P., Jeong, K. Il, Gonzalez, A.M., Saif, L.J., 2007. Transfer of  
 1065 maternal cytokines to suckling piglets: In vivo and in vitro models with implications for  
 1066 immunomodulation of neonatal immunity. *Vet. Immunol. Immunopathol.* 117, 236-248.  
 1067 doi:10.1016/j.vetimm.2007.02.013  
 1068 Olsson, C., Holmgren, S., 2011. Autonomic control of gut motility: a comparative view. *Auton.*  
 1069 *Neurosci.* 165, 80-101.  
 1070 Oste, M., De Vos, M., Van Haver, E., Van Brantegem, L., Thymann, T., Sangild, P., Weyns, A., Van  
 1071 Ginneken, C., 2010. Parenteral and enteral feeding in preterm piglets differently affects

1072 extracellular matrix proteins, enterocyte proliferation and apoptosis in the small  
 1073 intestine. Br. J. Nutr. 104, 989-997.

1074 Ostergaard, M.V., Bering, S.B., Jensen, M.L., Thymann, T., Purup, S., Diness, M., Schmidt, M.,  
 1075 Sangild, P.T., 2014. Modulation of intestinal inflammation by minimal enteral nutrition  
 1076 with amniotic fluid in preterm pigs. J. Parenter. Enteral Nutr. 38, 576-586.

1077 Pabst, R., Rothkotter, H., and Binns, R., 1991. Peyers-Patches Differ in Their Postnatal-  
 1078 Development, Lymphocyte Subsets and Entry of Lymphocytes and Lymphoblasts, in  
 1079 Frontiers of Mucosal Immunology, International Congress Series vol 939, Elsevier  
 1080 Science Publ B V, Amsterdam.

1081 Paßlack, N., Vahjen, W., Zentek, J., 2015. Dietary inulin affects the intestinal microbiota in sows  
 1082 and their suckling piglets. BMC Vet. Res. 11, 51. doi:10.1186/s12917-015-0351-7

1083 Paredes, S. P., Jansman, A. J., Verstegen, M.W., Awati, A.A., Buist, W., den Hartog, L. A., van Hees,  
 1084 H.M., Quiniou, N., Hendriks, W.H., Gerrits, W.J., 2012. Analysis of factors to predict piglet  
 1085 body weight at the end of the nursery phase. J. Anim. Sci. 90, 3243-3251.

1086 Payne, L.C., Marsh, C.L., 1962. Gamma Globulin Absorption in Baby Pig -Nonselective Absorption  
 1087 of heterologous globulins and factors influencing absorption time. J. Nutr. 76, 151-158.

1088 Patterson, R., Connor, M.L., Krause, D.O., Nyachoti, C.M., 2008. Response of piglets weaned from  
 1089 sows fed diets supplemented with conjugated linoleic acid (CLA) to an Escherichia coli  
 1090 K88+ oral challenge. Animal 2, 1303-1311. doi:10.1017/S1751731108002309

1091 Pieper, R., Zetzsche, A., Röhe, I., Tedin, L., Kröger, S., Vahjen, W., Zentek, J., 2016a, Bovine milk-  
 1092 based formula leads to early maturation-like morphological, immunological and  
 1093 functional changes in the jejunum of neonatal piglets. J. Anim. Sci. 94, 989-999.

1094 Pieper, R., Vahjen, W., Zentek, J., 2016b, Intestinal lactose and mineral concentration affect the  
 1095 microbial ecophysiology along the gastrointestinal tract of formula fed neonatal piglets. J.  
 1096 Anim. Sci. 94, 3786-3795.

1097 Pierzynowski, S.G., Weström, B.R., Svendsen, J., Svendsen, L., Karlsson, B.W., 1995, Development  
 1098 and regulation of porcine pancreatic function. Int. J. Pancreatol. 18, 81-94.

1099 Peng L., Li Z.-R., Green R.S., Holzman I.R., Lin J., 2009. Butyrate enhances the intestinal barrier by  
1100 facilitating tight junction assembly via activation of AMP-activated protein kinase in  
1101 Caco-2 cell monolayers. *J. Nutr.* 139, 1619–1625.

1102 Poroyko, V., White, J.R., Wang, M., Donovan, S., Alverdy, J., Liu, D.C., Morowitz, M.J., 2010. Gut  
1103 microbial gene expression in mother-fed and formula-fed piglets. *PLoS One* 5.  
1104 doi:10.1371/journal.pone.0012459

1105 Potten, C.S. 1997. Epithelial cell growth and differentiation. 2. Intestinal apoptosis. *Am. J. Physiol.*  
1106 *Gastrointest. Liver Physiol.* 273, G253-G257.

1107 Pluske, J.R., Hampson, D.J., Williams, I.H., 1997. Factors influencing the structure and function of  
1108 the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* 51, 215–236.

1109 Prims, S., Tambuyzer, B.R., Vergauwen, H., Huygelen, V., Van Cruchten, S., Van Ginneken, C.,  
1110 Casteleyn, C., 2016. Intestinal immune cell quantification and gram type classification of  
1111 the adherent microbiota in conventionally and artificially reared, normal and low birth  
1112 weight piglets. *Livest. Sci.* 185, 1-7.

1113 Quiniou, N., Gaudré, J.D., 2002. Variation of piglets' birth weight and consequences on  
1114 subsequent performance. *Livest. Prod. Sci.* 78, 63-70.

1115 Ramayo-Caldas, Y., Mach, N., Lepage, P., Levenez, F., Denis, C., Lemonnier, G., Leplat, J.-J., Billon, Y.,  
1116 Berri, M., Doré, J., Rogel-Gaillard, C., Estellé J., 2016, Phylogenetic network analysis  
1117 applied to pig gut microbiota identifies an ecosystem structure linked with growth traits.  
1118 *ISME J.* 10, 2973-2977.

1119 Rothkötter, H.J., and Pabst, R., 1989. Lymphocyte subsets in jejunal and ileal Peyer's patches of  
1120 normal and gnotobiotic minipigs. *Immunology* 67, 103–108.

1121 Salcedo, J., Frese, S.A., Mills, D.A., Barile, D., 2016. Characterization of porcine milk  
1122 oligosaccharides during early lactation and their relation to the fecal microbiome. *J.*  
1123 *Dairy Sci.* 99, 7733-7743.

1124 Sanderson, I.R, Walker, A., 1993. Uptake and transport of macromolecules by the intestine:  
1125 Possible role in clinical disorders (an update). *Gastroenterol.*, 104, 622-639.

1126 Sangild, P. T., Cranwell, P. D., Hilsted, L., 1992. Ontogeny of gastric function in the pig: acid  
 1127 secretion and the synthesis and secretion of gastrin. *Biol Neonate* 62, 363-372.

1128 Sangild, P. T., Foltmann, B., Cranwell, P. D., 1991. Development of gastric proteases in fetal pigs  
 1129 and pigs from birth to thirty six days of age. The effect of adrenocorticotropin (ACTH). *J*  
 1130 *Dev Physiol* 16: 229-238.

1131 Sangild, P.T., Schmidt, M., Elnif, J., Bjornvad, C.R., Westrom, B.R., Buddington, R.K., 2002. Prenatal  
 1132 development of gastrointestinal function in the pig and the effects of fetal esophageal  
 1133 obstruction. *Pediatr. Res.* 52, 416-424.

1134 Sangild, P.T., Siggers, R.H., Schmidt, M., Elnif, J., Bjornvad, C.R., Thymann, T., Grondahl, M.L.,  
 1135 Hansen, A.K., Jensen, S.K., Boye, M., Moelbak, L., Buddington, R.K., Westrom, B.R., Holst,  
 1136 J.J., Burrin, D.G., 2006. Diet- and colonization-dependent intestinal dysfunction  
 1137 predisposes to necrotizing enterocolitis in preterm pigs. *Gastroenterol.* 130, 1776-1792.

1138 Sangild, P.T., Thymann, T., Schmidt, M., Stoll, B., Burrin, D.G., Buddington, R.K., 2013. Invited  
 1139 review: the preterm pig as a model in pediatric gastroenterology. *J. Anim Sci.* 91, 4713-  
 1140 4729.

1141 Sangild, P.R., Trahair, J.F., Loftager, M.K., Fowden, A.L., 1999. Intestinal macromolecule  
 1142 absorption in the fetal pig after infusion of colostrum in utero. *Ped. Res.* 45, 595-602.

1143 Sawh, S.C., Deshpande, S., Jansen, S., Reynaert, C. J., Jones, P. M. 2016. Prevention of necrotizing  
 1144 enterocolitis with probiotics: a systematic review and meta-analysis. *Peer J.* 4:e2429.

1145 Schmidt, B., Mulder, I.E., Musk, C.C., Aminov, R.I., Lewis, M., Stokes, C.R., Bailey, M., Prosser, J.I.,  
 1146 Gill, B.P., Pluske, J.R., et al., 2011. Establishment of Normal Gut Microbiota Is  
 1147 Compromised under Excessive Hygiene Conditions. *Plos One* 6, e28284.

1148 Schutte, J.B., de Jong, J., van Weerden, E.J., Tamminga, S., 1992. Nutritional implications of L-  
 1149 arabinose in pigs. *Br. J. Nutr.* 68, 195-207. doi:10.1079/BJN19920077.

1150 Sepulveda, F.V., Smith, M.W., 1979. Inadequacy of the 'simple diffusion and mediated transport'  
 1151 model to describe amino acid influx into rabbit ileum. *J. Physiol.* 291, 58.

1152 Sinkora, M., Stepanova, K., Butler, J.E., Francis, D., Santiago-Mateo, K., Potockova, H., Karova, K.,  
 1153 and Sinkorova, J., 2011. Ileal Peyer's Patches Are Not Necessary for Systemic B Cell  
 1154 Development and Maintenance and Do Not Contribute Significantly to the Overall B Cell  
 1155 Pool in Swine. *J. Immunol.* 187, 5150–5161.

1156 Siggers, J., Ostergaard, M.V., Siggers, R.H., Skovgaard, K., Molbak, L., Thymann, T., Schmidt, M.,  
 1157 Moller, H.K., Purup, S., Fink, L.N., Frokiaer, H., Boye, M., Sangild, P.T., Bering, S.B., 2013.  
 1158 Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing  
 1159 enterocolitis in preterm neonates. *Am. J. Physiol Gastrointest. Liver Physiol* 304, G864-  
 1160 G875.

1161 Siggers, R.H., Siggers, J., Boye, M., Thymann, T., Molbak, L., Leser, T., Jensen, B.B., Sangild, P.T.,  
 1162 2008. Early administration of probiotics alters bacterial colonization and limits diet-  
 1163 induced gut dysfunction and severity of necrotizing enterocolitis in preterm pigs. *J. Nutr.*  
 1164 138, 1437-1444.

1165 Siggers, R.H., Siggers, J., Thymann, T., Boye, M., Sangild, P.T., 2011. Nutritional modulation of the  
 1166 gut microbiota and immune system in preterm neonates susceptible to necrotizing  
 1167 enterocolitis. *J. Nutr. Biochem.* 22, 511–521. doi:10.1016/j.jnutbio.2010.08.002

1168 Skrzypek, T., Piedra, J.L.V., Skrzypek, H., Kazimierczak, W., Szymanczyk, S.E., Zabielski, R., 2010.  
 1169 Changes in pig small intestinal absorptive area during the first 14 days of life. *Livest. Sci.*  
 1170 133, 53-56.

1171 Skrzypek, T., Valverde, Piedra, J.L., Skrzypek, H., Wolinski, J., Kazimierczak, W., Szymanczyk, S.,  
 1172 Pawlowska, M., Zabielski, R., 2005. Light and scanning electron microscopy evaluation of  
 1173 the postnatal small intestinal mucosa development in pigs. *J. Physiol. Pharmacol.* 56  
 1174 Suppl 3, 71-87.

1175 Smith, M.W., Peacock, M.A., 1980. Anomalous replacement of foetal enterocytes in the neonatal  
 1176 pig. *Proc. Royal Soc. Lond. B.* 206, 411-420.

1177 Snoeck, V., Huyghebaert, N., Cox, E., Vermeire, A., Saunders, J., Remon, J.P., Verschooten, F.,  
 1178 Goddeeris, B.M. 2004. Gastrointestinal transit time of nondisintegrating radio-opaque  
 1179 pellets in suckling and recently weaned piglets. *J. Control. Release* 94, 143-153.  
 1180 Starke, I.C., Pieper, R., Neumann, K., Zentek, J., Vahjen, W., 2013. Individual responses of mother  
 1181 sows to a probiotic *Enterococcus faecium* strain lead to different microbiota composition  
 1182 in their offspring. *Benef. Microbes* 4, 345–356. doi:10.3920/BM2013.0021  
 1183 Sun, J., Hayward, C., Shinde, R., Christenson, R., Ford, S.P., and Butler, J.E., 1998. Antibody  
 1184 repertoire development in fetal and neonatal piglets. I. Four V-H genes account for 80  
 1185 percent of V-H usage during 84 days of fetal life. *J. Immunol.* 161, 5070–5078.  
 1186 Sty, A.C., Sangild, P.T., Skovgaard, K., Thymann, T., Bjerre, M., Chatterton, D.E., Purup, S., Boye, M.,  
 1187 Heegaard, P.M., 2016. Spray dried, pasteurised bovine colostrum protects against gut  
 1188 dysfunction and inflammation in preterm pigs. *J. Pediatr. Gastroenterol. Nutr.* 63, 280-  
 1189 287.  
 1190 Svendsen J., Weström, B.R., Olsson, A.-C., Svendsen, L.S., Karlsson, B.W., 1990. Environmental and  
 1191 husbandry factors affecting the acquisition of passive immunity in the suckling pig.  
 1192 *World Rev. Anim. Prod.* 25, 11-19.  
 1193 Takeda, T., Sakata, M., Minekawa, R., Yamamoto, T., Hayashi, M., Tasaka, K., Murata, Y., 2004.  
 1194 Human milk induces fetal small intestinal cell proliferation involvement of a different  
 1195 tyrosine kinase signaling pathway from epidermal growth factor receptor. *J. Endocrinol.*  
 1196 181, 449-457.  
 1197 Tao, N., Ochonicky, K.L., German, J.B., Donovan, S.M., Lebrilla, C.B., 2010. Structural  
 1198 determination and daily variations of porcine milk oligosaccharides. *J. Agric. Food Chem.*  
 1199 58, 4653-4659.  
 1200 Thompson, C.L., Wang, B., Holmes, A.J., 2008. The immediate environment during postnatal  
 1201 development has long-term impact on gut community structure in pigs. *ISME J.* 2, 739–  
 1202 48. doi:10.1038/ismej.2008.29



1203 Thum, C., Cookson, A.L., Otter, D.E., McNabb, W.C., Hodgkinson, A.J., Dyer, J., Roy, N.C., 2012. Can  
 1204 nutritional modulation of maternal intestinal microbiota influence the development of  
 1205 the infant gastrointestinal tract? *J. Nutr.* 142, 1921-1928.

1206 Thymann, T., Moller, H.K., Stoll, B., Stoy, A.C., Buddington, R.K., Bering, S.B., Jensen, B.B., Olutoye,  
 1207 O.O., Siggers, R.H., Molbak, L., Sangild, P.T., Burrin, D.G., 2009. Carbohydrate maldigestion  
 1208 induces necrotizing enterocolitis in preterm pigs. *Am. J. Physiol. Gastrointest. Liver*  
 1209 *Physiol.* 297, G1115-G1125.

1210 Thymann, T., 2016. Endocrine regulation of gut maturation in early life in pigs. *Domest. Anim*  
 1211 *Endocrinol.* 56, S90-93.

1212 Trahair, J.F., Sangild, P.T., 2000. Fetal organ growth in response to oesophageal infusion of  
 1213 amniotic fluid, colostrum, milk or gastrin-releasing peptide: a study in fetal sheep.  
 1214 *Reprod. Fertil. Dev.* 12, 87-95.

1215 Tuchscherer, M., Puppe, B., Tuchscherer, A., Tiemann, U., 2000. Early identification of neonates at  
 1216 risk: traits of newborn piglets with respect to survival. *Theriogenol.* 54, 371-388.

1217 Vahjen, W., Taras, D., Simon, O., 2007. Effect of the probiotic *Enterococcus faecium* NCIMB10415  
 1218 on cell numbers of total *Enterococcus* spp., *E. faecium* and *E. faecalis* in the intestine of  
 1219 piglets. *Curr. Issues Intestinal Microbiol.* 8, 1-8.

1220 Vanderhaeghe, C., Dewulf, J., Jourquin, J., De, K. A., Maes, D., 2011. Incidence and prevention of  
 1221 early parturition in sows. *Reprod. Domest. Anim* 46, 428-433.

1222 Van Ginneken, C., 2012. The gastrointestinal system. In: *The minipig in biomedical research*. ED.  
 1223 Dayan, A., Hastings, K., McAnulty, P., Ganderup, N.-C., CRC Press. p211-236.

1224 Van Ginneken, C., Van Meir, F., Sys, S., Weyns, A., 2001. Developmental changes in heme-  
 1225 oxygenase-2 and bNOS expression in enteric neurons in the pig duodenum. *Auton.*  
 1226 *Neurosci.* 91, 16-25.

1227 Van Ginneken, C., Van Meir, F., Sys, S., Weyns, A., 2002. Stereologic characteristics of pig small  
 1228 intestine during normal development. *Dig. Dis. Sci.* 47, 868-878.

1229 Van Ginneken, C., Weyns, A., 2004. A stereological evaluation of secretin and gastric inhibitory  
 1230 peptide-containing mucosal cells of the perinatal small intestine of the pig. *J. Anat.* 205,  
 1231 267-275.

1232 Vega-Lopez, M., Telemo, E., Bailey, M., Stevens, K., and Stokes, C., 1993. Immune cell distribution  
 1233 in the small-intestine of the pig - immunohistological evidence for an organized  
 1234 compartmentalization in the lamina propria. *Vet. Immunol. Immunopathol.* 37, 49-60.

1235 Vega-Lopez, M., Bailey, M., Telemo, E., and Stokes, C., 1995. Effect of early weaning on the  
 1236 development of immune cells in the pig small-intestine. *Vet. Immunol. Immunopathol.*  
 1237 44, 319-327.

1238 Wang, W., Degroote, J., Van Ginneken, C., Van Poucke, M., Vergauwen, H., Dam, T.M.T.,  
 1239 Vanrompay, D., Peelman, L.J., De Smet, S., Michiels, J., 2016. Intrauterine growth  
 1240 restriction in neonatal piglets affects small intestinal mucosal permeability and mRNA  
 1241 expression of redox-sensitive genes. *Faseb J* 30, 863-873.

1242 Wang, T., Huo, Y.J., Shi, F., Xu, R.J., Hutz, R.J. 2005. Effects of intrauterine growth retardation on  
 1243 development of the gastrointestinal tract in neonatal pigs. *Biol. Neonate* 88, 66-72.

1244 Wang, M., Radlowski, E.C., Jr, G.C.F., Gaskins, H.R., Donovan, S.M., 2013. Mode of delivery and  
 1245 early nutrition modulate microbial colonization and fermentation products in neonatal  
 1246 piglets. *J. Nutr.* 143, 795-803. doi:10.3945/jn.112.173096.795

1247 Wang, X., Wu, W., Lin, G., Wu, G., Wang, J., 2010. Temporal proteomic analysis reveals continuous  
 1248 impairment of intestinal development in neonatal piglets with intrauterine growth  
 1249 restriction. *J. Proteome Res.* 9, 924-935.

1250 Wang, T., Xu, R.J., 1996. Effects of colostrum feeding on intestinal development in newborn pigs.  
 1251 *Biol. Neonate* 70, 339-348.

1252 Wangsness, P.J., Soroka, G.H., 1978. Effect of energy concentration of milk on voluntary intake of  
 1253 lean and obese piglets. *J. Nutr.* 108, 595-600.

1254 Willemen, S., Che, L., De Vos, M., Huygelen, V., Tambuyzer, B., Casteleyn, C., Van Cruchten, S.,  
 1255 Zhang, K., Van Ginneken, C., 2012. Perinatal growth restriction is not related to higher

1256 intestinal distribution and increased serum levels of 5-hydroxytryptamin in piglets. J.  
1257 Anim. Sci. 90, 305-307.

1258 Willemen, S.A., De Vos, M., Huygelen, V., Fransen, E., Tambuyzer, B.R., Casteleyn, C., Van Cruchten,  
1259 S., Van Ginneken, C., 2013. Ghrelin in the gastrointestinal tract and blood circulation of  
1260 perinatal low and normal weight piglets. *Animal* 7, 1978-1984.

1261 Wilson, A.D., Haverson, K., Southgate, K., Bland, P.W., Stokes, C.R., and Bailey, M., 1996.  
1262 Expression of major histocompatibility complex class II antigens on normal porcine  
1263 intestinal endothelium. *Immunology* 88, 98-103.

1264 Wilson, S., Norton, P., Haverson, K., Leigh, J., and Bailey, M., 2005. Development of the palatine  
1265 tonsil in conventional and germ-free piglets. *Dev. Comp. Immunol.* 29, 977-987.

1266 Wilson, S., Norton, P., Leigh, J., and Bailey, M., 2007. Early, microbially driven follicular reactions  
1267 in the neonatal piglet do not contribute to expansion of the immunoglobulin heavy chain  
1268 V-D-J repertoire. *Vet Immunol Immunopathol* 118, 105-112.

1269 Wilson, A.D., Stokes C.R., Bourne, F.J., 1989. Effect of age on absorption and immune responses to  
1270 weaning or introduction of novel dietary antigens. *Res. Vet. Sci.* 46, 180-186.

1271 Wiyaporn, M., Thongsong, B., Kalandakanond-Thongsong, S., 2013. Growth and small intestine  
1272 histomorphology of low and normal birth weight piglets during the early suckling period.  
1273 *Livest. Sci.* 158, 215-222.

1274 Weström, B.R., Ohlsson, B.G., Svendsen, J., Tagesson, C., Karlsson, B.W., 1985. Intestinal  
1275 transmission of macromolecules (BSA and FITC-labelled dextrans) in the neonatal pig.  
1276 Effect of colostrum, protease inhibitors and protein on the efficiency of transmission.  
1277 *Biol. Neonate*, 47, 359-366.

1278 Weström, B.R., Svendsen, J., Ohlsson, B.G., Tagesson, C., Karlsson, B.W., 1984. Intestinal  
1279 transmission of macromolecules (BSA and FITC-labelled dextrans) in the neonatal pig.  
1280 Influence of age of piglet and molecular weight of markers. *Biol. Neonate*, 46, 20-26.

1281 Wolinski, J., Biernat, M., Guilloteau, P., Westrom, B.R., Zabielski, R., 2003. Exogenous leptin  
 1282 controls the development of the small intestine in neonatal piglets. *J. Endocrinol.* 177,  
 1283 215-222.

1284 Wooding, F.B.P., Smith, M.W., Craig, H., 1978. The ultrastructure of the neonatal pig colon. *Am. J.*  
 1285 *Anat.* 152, 269-285

1286 Wright, A.B., McKelvey, G.M., Wood, A.K.W., Post, E.J. 1998. Sonographic observations of the  
 1287 gastroduodenal junction in neonatal piglets. *Ultrasound Med. Biol.* 24, 1337-1344.

1288 Xiao, L., Estellé, J., Kiilerich, P., Ramayo-Caldas, Y., Xia, Z., Feng, Q., Liang, S., Pedersen, A.Ø.,  
 1289 Kjeldsen, N.J., Liu, C., Maguin, E., Doré, J., Pons, N., Le Chatelier, E., Prifti, E., Li, J., Jia, H.,  
 1290 Liu, X., Xu, X., Ehrlich, S.D., Madsen, L., Kristiansen, K., Rogel-Gaillard, C., Wang, J., 2016. A  
 1291 reference gene catalogue of the pig gut microbiome. *Nat. Microbiol.*, *in press*. doi:  
 1292 10.1038/nmicrobiol.2016.161.

1293 Xu, R. J., Cranwell, P. D., 1990. Development of gastric acid secretion in pigs from birth to thirty  
 1294 six days of age : The response to pentagastrin. *J. Dev. Physiol.* 13, 315-326.

1295 Xu, R. J., Mellor, D.J., Birtles, M.J., Reynolds, G.W., Simpson, H.V. 1994. Impact of intrauterine  
 1296 growth retardation on the gastrointestinal tract and the pancreas in newborn pigs. *J. Ped.*  
 1297 *Gastroent. Nutr.* 18, 231-240.

1298 Xu, R.J., Sangild, P.T., Zhang, Y.Q., Zhang, S.H., 2002. Bioactive compounds in porcine colostrum  
 1299 and milk and their effects on intestinal development in neonatal pigs. In: Zabielski, R.,  
 1300 Gregory, P.C., Westrom, B., (Eds.), *Biology of the Intestine in Growing Animals*, Elsevier,  
 1301 Amsterdam, pp. 169-192.

1302 Xu, R.J., Tungthanathanich, P., Birtles, M.J., Mellor, D.J., Reynolds, G.W., Simpson, H.V., 1992.  
 1303 Growth and morphological changes in the stomach of newborn pigs during the first three  
 1304 days after birth. *J. Dev. Physiol.* 17, 7-14.

1305 Yasuda, M., Jenne, C.N., Kennedy, L.J., and Reynolds, J.D., 2006. The sheep and cattle Peyer's patch  
 1306 as a site of B-cell development. *Vet. Res.* 37, 401-415.

1307 Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris,  
 1308 M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., Heath, A.C., Warner, B., Reeder, J.,  
 1309 Kuczynski, J., Caporaso, J.G., Lozupone, C.A., Lauber, C., Clemente, J.C., Knights, D., Knight,  
 1310 R., Gordon, J.I., 2012, Human gut microbiome viewed across age and geography. *Nature*,  
 1311 486, 222-227.

1312 Yen, T.H., Wright, N.A., 2006. The gastrointestinal tract stem cell niche. *Stem Cell Rev.* 2, 203-212.

1313 Zhang, H.Z., Malo, C., Buddington, R.K., 1997. Suckling induces rapid intestinal growth and  
 1314 changes in brush border digestive functions of newborn pigs. *J. Nutr.* 127, 418-426.

1315 Ziegler, T.R., Estivariz, C.F., Jonas, C.R., Gu, L.H., Jones, D.P., Leader, L.M., 1999. Interactions  
 1316 between nutrients and peptide growth factors in intestinal growth, repair, and function.  
 1317 *J. Parenter. Enteral Nutr.* 23, S174-S183.

1318 Ziegler, T.R., Evans M.E., Fernandez-Estivariz, C., Jones, D.P., 2003. Trophic and cytoprotective  
 1319 nutrition for intestinal adaptation, mucosal repair, and barrier function. *Ann.Rev. Nutr.*  
 1320 23, 229-261.

Table 1. Overview of developmental changes after birth in pigs

| Developmental changes after birth   | References  |
|---|---|
| <i>Structural aspects</i>   |   |
| Massive growth of the stomach by hypertrophy and mainly hyperplasia   | Lindemann et al. 1986   |
| Shift in enterocyte types   | Smith and Peacock, 1980, Klein, 1989  |
| Postnatal enlargement of the intestinal mucosa, changes in villus length and crypt depth, accompanied by changes in the shape of the villi  | Wolinski et al., 2003; Godlewski et al., 2007; Skrzypek et al, 2005; Skrzypek et al, 2010 |
| Uptake of macromolecules to the enterocytes by endocytosis which ceases at a certain moment due to intestinal closure                       | Brambell, 1970; Baintner, 1986  |
| Bioactive substances in the colostrum and milk stimulate intestinal mucosal proliferation and facilitate the closure of the small intestine | Takeda et al., 2004   |
| Morphological changes of colonocytes, with disappearance of villi-like structures   | Cremaschi et al. 1979; Xu et al. 1992a  |
| <i>Functional aspects</i>   |   |
| Gastric acid secretion is low at birth but increases rapidly during the first week of life  | Xu and Cranwell 1990, Sangild et al. 1991; Sangild et al. 1992                            |
| Activities of the brush border peptidases are relatively high at birth and then decrease with age in suckling pigs                          | Le Huerou-Luron, 2002   |
| Shift in expression of the enterocyte brush border disaccharidases  | Le Huërou-Luron 2002  |
| Lactase activity decreases with age, while maltase and sucrase activities increase  | Le Huerou-Luron 2002  |
| As the milk clots in the stomach, a period of inhibition of motility is established, followed by a slow phase of emptying                   | Decuypere et al., 1986  |
| During aging, a faster small intestinal transit is observed   | Huygelen et al., 2015   |
| <i>Gut immune system</i>  |   |
| The newborn piglet are born hypogammaglobulinaemic and must acquire passive immunity (IgG) via mammary secretions for their survival        | Bailey et al, 2005  |
| Peyer's patches are present at birth and contain very small, primordial follicles and almost no T-cells                                     | Makala et al, 2000  |
| While the size of B-cell and T-cell compartments expands, function remains limited for several weeks  | Barman et al, 1997; Makala et al, 2000  |

|   |  |
|---|--|
| While piglets under 6 weeks can make antigen-specific responses, the quality of the response may be limited compared to older animals   | Wilson et al, 2005   |
| Antigen-presenting cells appear in the intestinal mucosa during the first two weeks of life   | Bianchi et al, 1992; Vega-Lopez et al, 1995  |
| CD4 T-cells appear in the mucosa during weeks three and four, and CD8 T-cells in the epithelium start to appear from four to 6 weeks old  | Bianchi et al, 1992; Vega-Lopez et al, 1995  |
| <i>Gut colonization</i>   |  |
| The establishment of the intestinal microbiome of pigs occurs early in life and is dependent on events such as environmental or dietary changes, or medical intervention  | Koenig et al., 2011; Yatsunenکو et al., 2012; Thompson et al., 2008; Schmidt et al., 2011; Starke et al., 2013 |
| The very early colonizers between birth and 2 days of age are mainly members of the genera <i>Escherichia</i> , <i>Clostridium</i> , <i>Fusobacterium</i> , <i>Streptococcus</i> and <i>Enterococcus</i> , whereas <i>Lactobacillus</i> , <i>Bacteroides</i> , <i>Prevotella</i> and <i>Ruminococcus</i> increase in abundance afterwards during undisturbed colonization processes | Bian et al., 2016; Kubasova et al., 2017   |
| Pre-weaning interventions in husbandry and diet have been shown to affect the establishment of intestinal microbiome and the development of the immune system   | Inman et al, 2010b; Lewis et al, 2012  |
| <i>Colostrum and milk</i>   |  |
| A fast drop in the protein concentration of the milk, mainly due to a decrease in immunoglobulins, is observed, while the concentration of the energetic molecules, i.e. lipids and lactose increase  | Devillers and Lessard, 2007  |
| About 30 porcine milk oligosaccharides have been identified, and changes in their composition also occur throughout lactation   | Tao et al., 2010; Salcedo et al., 2016   |